January 2002
Volume 3, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Control Conjugates: The Perfect Companion for Targeted Toxins

Dr. Douglas Lappi, Chief Scientific Officer, Advanced Targeting Systems

The field of targeted toxins has made enormous strides in the years since Advanced Targeting Systems introduced the first research targeted toxin in 1994. We now offer 16 different targeted toxins, with more on the way. The number of important papers in high impact journals continues to rise, and the sophistication of the studies is very impressive.

Much of the research using targeted toxins has been dedicated to characterizing the lesions caused by these molecules. We studied this important work to understand where ATS could be most helpful. The result of our analysis is the offering this year of new control molecules that chemically resemble the targeted toxins, but are not targeted to any cell type.

Saporin alone (not conjugated) has been used by some researchers as a control. But without a stand-in for the targeting agent, there are fundamental differences in physiochemical structure. The new controls solve this problem. Some researchers have included the targeting agent mixing the antibody or peptide with saporin. While this has the components of the targeted toxin, it does not include the structural features of the conjugation chemistry. The new controls solve this difficulty.

(continued on page 6)

SFN 2001 Abstract Award Winner

Doug Lappi congratulates Mary Ann Greco as she proudly shows off her ATS cap and mug, some of her prizes for presenting the winning abstract using an ATS product at the 2001 SFN meeting in San Diego. There were 62 competing research presentations--a record number for ATS research products.

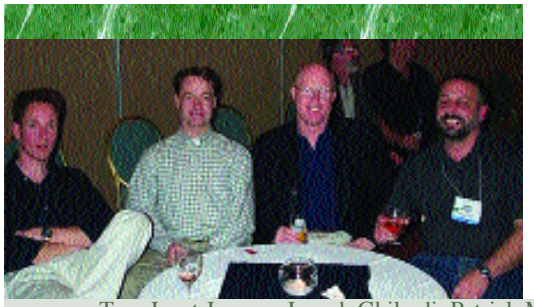
Hypocretin B-Saporin Lesions of the Brainstem Increase REM Sleep at Night
CA Blanco-Centurion, RJ Salin-Pascual, D Gerashchenko, MA Greco, PJ Shiromani.
Harvard Medical School & UNAM, Mexico City

This year's award-winning abstract featured orexin-SAP (Cat #IT-20) and anti-DBH-SAP (Cat #IT-03). This poster described data on the role of the locus coeruleus in sleep regulation.

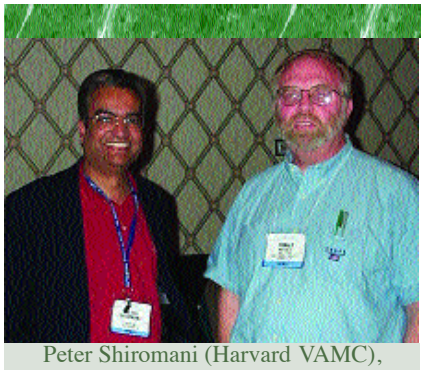
Mary Ann Greco was the presenting author and will be contributing the cover story in *Targeting Trends* April 2002 issue. She will discuss the fascinating and surprising results. Congratulations, Mary Ann!

ATS Customers Appreciated at Party

A good time was had by all at the Customer Appreciation party hosted by Advanced Targeting Systems at the 2001 Society for Neuroscience meeting. The party offered the perfect opportunity for researchers to talk about the latest research techniques while enjoying good food and lively libations. There was also plenty of time to kick back and laugh with friends after another long day at the meeting.



Top: Joost Jongen, Joseph Ghilardi, Patrick Mantyh, Sergey Khasabov (Univ Minnesota).
Right: William Truitt, Lique Coolen, Margaret Balfour (Univ Cinncinati), Doug Lappi (ATS)



Peter Shiromani (Harvard VAMC), Ron Wiley (Vanderbilt DVAMC)

Ann Schreihofer (Med Coll Georgia), Derek Schreihofer, Bob Speth (Washington State)

Doug Lappi (ATS), Darlene Martineau (Doug's wife), Bob Yezierski (Univ Florida)

Denise Higgins, Brian Russell, Kristie Majer, Doug Lappi, Matt Kohls (ATS)

Eric Barker, (Purdue University), Craig Wrenn (NIH, NIMH)

Matt Kohls, Cynthia Wilson, Kristie Majer, Brian Russell (ATS)

Upcoming Events

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

Experimental Biology
New Orleans, LA • April 20-24

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Colocalization of μ-opioid Receptors and Activated G-proteins in Rat Cingulate Cortex

Vogt LJ, Sim-Selley LJ, Childers SR, Wiley RG, and Vogt BA
J Pharmacol Exper Ther 299:840-848, 2001

The anterior cingulate cortex (ACC) is a primary site of opiate drug action, and much of this activity is associated with the μ-opioid receptor (MOR). The mechanisms by which MOR regulates pain in the ACC are not well understood. Using anti-DBH-SAP (7 μg into left lateral ventricle in rat; Cat. #IT-03) the authors mapped MOR activity in the ACC and evaluated the histochemical and behavioral relationships between MOR binding and μ-receptor-activated G-proteins after lesioning.

Selective Immunolesions of Cholinergic Neurons in Mice: Effects on Neuroanatomy, Neurochemistry, and Behavior

Berger-Sweeney J, Stearns NA, Murg SL, Floerke-Nashner LR, Lappi DA, Baxter MG
J Neurosci 21(20):8164-8173, 2001

192-Saporin (Cat. #IT-01) has long been an effective agent for elimination of cholinergic neurons in the basal forebrain of rats. Until the development of mu p75-SAP (Cat. #IT-16) there was no equivalent agent for use in mice. The authors tested mu p75-SAP *in vitro* and *in vivo* (1.8-3.6 μg in right lateral ventricle), using cytotoxic, histochemical, and behavioral assays. The data shows that mu p75-SAP is a highly selective and efficacious lesioning agent for cholinergic neurons in the mouse. The authors conclude that mu p75-SAP will be a powerful tool to use in combination with genetic modification to investigate cholinergic damage in mouse models of Alzheimer's disease.

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

Extensive Immunolesions of Basal Forebrain Cholinergic System Impair Offspring Recognition in Sheep

Ferreira G, Meurisse M, Gervais R, Ravel N, Levy F
Neurosci 106(1):103-115, 2001

Through the use of 192-Saporin (Cat. #IT-01) the association of basal forebrain cholinergic neurons to learning instrumental tasks has been well established in the rat. The authors wished to examine whether these neurons were also associated with social learning tasks, such as offspring recognition in sheep. Using ME20.4-SAP (Cat. #IT-15) the basal forebrain cholinergic neurons of sheep were lesioned by intraventricular bilateral injections (150 μg). The results demonstrate that these neurons contribute to visual discrimination learning, and are involved in formation of lamb recognition memory.

Dissociation between the Attentional Functions Mediated via Basal Forebrain Cholinergic and GABAergic Neurons

Burk JA, Sarter M
Neurosci 105(4):899-909, 2001

The specificity and efficacy of 192-Saporin (Cat. #IT-01) has allowed the extensive investigation of cortical cholinergic inputs in attentional functions. Little is known about the function of non-cholinergic neurons because of the lack of a specific tool to eliminate these projections. The authors injected 192-Saporin (0.1 μg/0.5 μl bilateral infusions) into rats and compared performance to rats treated with ibotenic acid to eliminate GABAergic neurons in attention performance tasks. While the ibotenic acid lesions were not as specific as those produced by 192-Saporin, the data suggest a role for the basal forebrain GABAergic neurons in attentional functions.

Novel Method for Localized, Functional Sympathetic Nervous System Denervation of Peripheral Tissue Using Guanethidine

Demas GE, Bartness TJ
J Neurosci Meth 112:21-28, 2001

Sympathectomy, or surgical interruption of sympathetic nerve pathways, is an important technique in the analysis of the sympathetic nervous system. The authors investigate and compare several different methods of performing a sympathectomy in hamsters, including surgery, chemical, and immunotoxic lesions using anti-DBH-SAP (ten 2-μl injections, at either 0.65 μg/μl or 0.325 μg/μl, into inguinal white adipose tissue; Cat. #IT-03).

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Macrophage-Derived IL-18-Mediated Intestinal Inflammation in the Murine Model of Crohn's Disease

Kanai T, Watanabe M, Okazawa A, Sato T, Yamazaki M, Okamoto S, Ishii H, Totsuka T, Iiyama R, Okamoto R, Ikeda M, Kurimoto M, Takeda K, Akira S, and Hibi T
Gastroenterol 121:875-888, 2001

Crohn's disease is an inflammatory bowel disease that is associated with several changes in the immune system, including an increased number of infiltrating macrophages. These macrophages release a variety of cytokines that are responsible for inflammation. The authors investigated the role of these macrophages in a mouse model by eliminating them with Mac-1-SAP (20 μg parenterally in tail vein; Cat. #IT-06). Seven days after treatment, mice showed no evidence of intestinal inflammation. These data demonstrate the role of macrophages in the development of inflammatory bowel conditions.

The Effects of Manipulations of Attentional Demand on Cortical Acetylcholine Release

Himmelheber AM, Sarter M, Bruno JP
Cogn Brain Res 12:353-370, 2001

Cortical cholinergic afferents from the basal forebrain are suspected to be involved in attentional tasks. Regulatory impairment of these afferents has been hypothesized to contribute to attentional deficits seen in conditions as diverse as Alzheimer's disease and schizophrenia. The authors have previously shown that 192-Saporin (Cat. #IT-01) lesions result in severe impairments in tasks requiring sustained attentional processing. In these experiments the authors suggest that cell response is dependent on the level of demand. They demonstrate that removal of p75+ cells (0.5 μg/μl bilaterally infused into the nucleus basalis region in rat) impairs sustained attentional performance, but does not impact low-demand task performance.

Long-Term Intrathecal Catheterization in the Rat

Jasmin L, Ohara PT
J Neurosci Meth 110:81-89, 2001

The authors have developed a method that allows repeated administration of drugs with minimal stress to an experimental animal. To test the efficacy of this intrathecal catheter, they injected anti-DBH-SAP (5 μg; Cat. #IT-03,) and investigated the noradrenergic denervation of the spinal cord. All animals treated with anti-DBH-SAP showed extensive loss of spinal noradrenergic ennervation. Even three months after catheter implantation, the elimination of noradrenergic neurons in the spinal cord could be produced. This indicates the intrathecal catheter is an effective tool for the study of multiple-dose drug delivery.

Differential Changes in Rat Cholinergic Parameters Subsequent to Immunotoxic Lesion of the Basal Forebrain Nuclei

Waite JJ, Chen AD
Brain Res 918:113-120, 2001

192-Saporin (Cat. #IT-01) is used extensively to eliminate the cholinergic neurons of the basal forebrain in rats. Waite and Chen compare the degree of loss between 192-Saporin (6 or 8.2 μg in 10 μl into left lateral ventricle) and control (Saporin, 1.82 μg into left lateral ventricle; Cat. #PR-01) using three methods: Assay of post mortem choline acetyltransferase activity, *in vivo* microdialysis of extracellular acetylcholine (ACh), and *in vivo* assessment of the rate of ACh synthesis. The infusion of saporin alone had no effect. After fifteen weeks, the authors report compensation of cholinergic activity in lesioned animals occurs in the hippocampus, but not in the frontal cortex as determined by measurement of the rate of ACh synthesis.

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

Targeting Talk: *Toxin Safety*

by Dr. Ronald G. Wiley

Q: We're submitting a protocol to our IACUC to use IB4-SAP. We plan to inject the targeted toxin and then sacrifice the animal ten days later. What, if any, are the safety issues here?

A: The only danger to lab personnel from IB4-SAP would be accidental self-injection, and even then, at the doses typically used in rats, it would only produce very localized effects at the injection site.

Once injected into animals, the agent is rapidly rendered inaccessible to anyone else by binding, internalization and eventual catabolism. It is extremely unlikely that intact toxin would ever be excreted or recoverable from the rats. The components of the toxin, IB4 and saporin, by themselves are no toxic threat. We use no special precautions with such rats except appropriate care for whatever neurologic deficits they develop, i.e. foot drop, autotomy, etc.

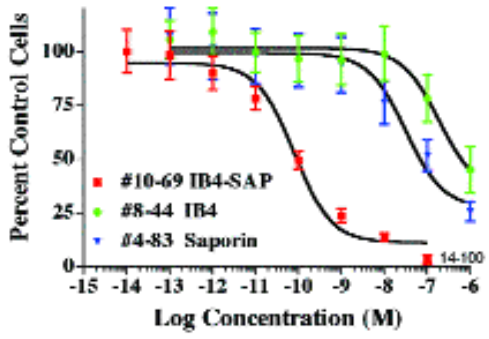
One caveat: To the best of my knowledge the above statements are accurate, but I do not know of any experimental data that directly addresses the issues. I base my comments on our long experience with similar agents including ricin and volkensin which are much more toxic and unstable.

Safety Instructions

Good laboratory technique must be employed for the safe handling of this product. This requires observation of the following practices:

1. Wear appropriate laboratory attire, including lab coat, gloves and safety glasses.
2. Do not pipet by mouth, inhale, ingest or allow product to come into contact with open wounds. Wash thoroughly any part of the body which comes into contact with the product.
3. Avoid accidental autoinjection by exercising extreme care when handling in conjunction with any injection device.
4. This product is intended for research use by qualified personnel only. It is not intended for use in humans or as a diagnostic agent. Advanced Targeting Systems is not liable for any damages resulting from the misuse or handling of this product.

For disposal: autoclave, or expose to 0.2 M NaOH, materials that come into contact with the toxin.



IB4-SAP(Cat. # IT-10)

a chemical conjugate of the B4 lectin [from Griffonia (Bandeiraea) simplicifolia*] and the ribosome-inactivating protein saporin*

IB4-SAP specifically eliminates α-D-Galactosyl-positive cells. Excellent for the study of pain transmission

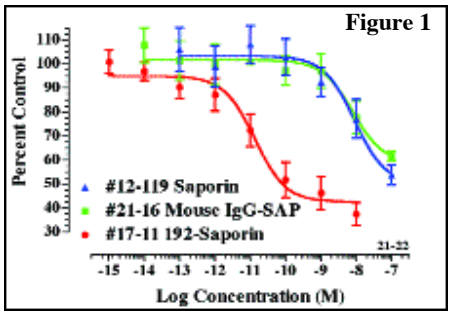
Control Conjugates: The Perfect Companion

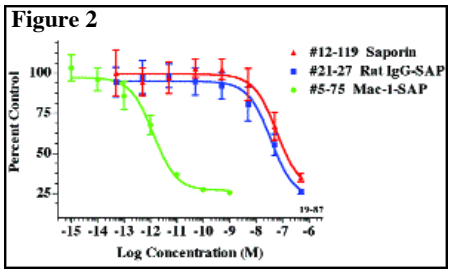
(continued from page 1)

ATS targeted toxins fall into three categories: immunotoxins, ligand toxins and second immunotoxins. To provide researchers with more verifiable targeting tools, each of these now has a dedicated control.

NEW! Immunotoxins: This category can be further divided into those with antibodies that are based on mouse monoclonals (192-Saporin, OX7-SAP, anti-DBH-SAP, ME20.4-SAP), and those with antibodies based on rat monoclonals (Mac-1-SAP, mu p75-SAP).

For the mouse monoclonals, we offer Mouse-IgG-SAP (Cat# IT-18), produced by conjugation of mouse IgG to saporin. Mouse IgG replaces the murine monoclonal IgG that performs the specific targeting, and saporin is used in both. The molecular structure of the two is similar, even to the chemistry of conjugation. The only difference is the replacement of the targeting agent with mouse IgG that has no target. Figure 1 shows the same lack of targeted cytotoxicity as saporin alone and approximately 1000-fold less than targeted material.

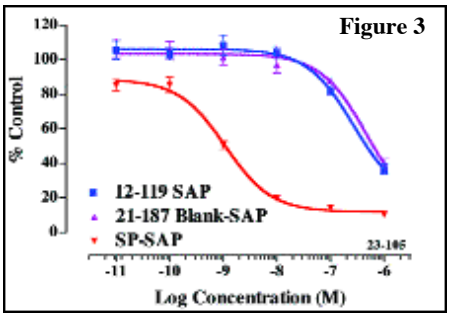




The rat immunotoxins also have a negative control. Rat IgG-SAP, Cat# IT-17, is made with rat IgG coupled to saporin with the same chemistry. Figure 2 shows that rat IgG-SAP has no more cytotoxicity to cells than saporin alone, while the targeted toxin is more than four orders of magnitude more toxic.

NEW! For the second immunotoxins (Mab-ZAP, Rab-ZAP), use Goat IgG-SAP, Cat# IT-19. It works on the same easy principle as Mouse IgG-SAP and Rat IgG-SAP.

NEW! Ligand-toxins: Blank-SAP is constructed from a nonsense peptide, a re-arranged alpha melanocyte-stimulating hormone. This sequence contains amino acids common to the ligands of G protein-coupled receptors, but has no known homology. Like all of our peptide-toxins, it has a 1:1 molar ratio of saporin to peptide, and is void of any free peptide or non-conjugated saporin. It's a perfect match to the peptide ligand-toxins SP-SAP, SSP-SAP, orexin-SAP, dermorphin-SAP, and CRF-SAP. Figure 3 is an illustration of Blank-SAP "shooting blanks" relative to a targeted toxin. The targeted toxin is more than two orders of magnitude more potent than Blank-SAP or saporin alone.



The great thing about these new controls is they are ready to use. You don't have to make strange calculations to figure how much of each component to add; you just use the same amount of control as you do the targeted toxin, and that's it! Couldn't be much simpler.

Targeting Ticklers

Excerpts from "The Ultimate Scientific Dictionary"

As we took notes, our anatomy instructor labored through a lecture on the way nerve cells transmit impulses. "Who can tell me how these cells communicate with one another?" he asked, expecting someone to explain the phenomenon of neurotransmission. After a few muffled whispers, one student finally spoke up. "With cellular phones?"

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: HEPARAN FLASK SYRINGE ROBOTICS

Answer: What the scientist forgot to add to the formula --- A PINCHOFSALT

WINNERS: Lynn Young, RW Johnson PRI * Dr. Eduardo Colombari, Unifesp-Epm * Dr. Robert Speth, Washington State University

Targeting Tools: Featured Products

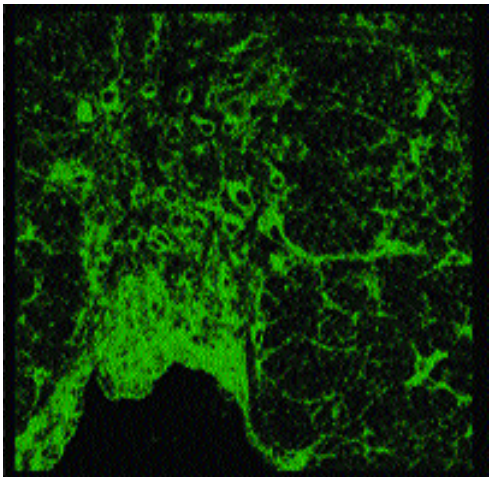
Serotonin Transporter Antibody

Advanced Targeting Systems announces a new reagent for the study of the serotonergic systems—a monoclonal antibody to the serotonin transporter. This murine monoclonal is made with a peptide from an extracellular domain of rat serotonin re-uptake transporter (SERT), and thus is able to attach to cells that express SERT. Homology with the human form is very high, but very low for other transporters such as the norepinephrine transporter (NET) and dopamine transporter (DAT). Figure 1 shows FACS analysis of human platelets, which express the transporter. There is a strong shift with antibody labeled with FITC-second antibody conjugate. FACS analysis also shows no interaction with cells expressing NET or DAT, as is expected from the homology cited above. Figure 2 demonstrates excellent immunostaining by the antibody of the rat raphe nucleus, a major site of SERT expression.

This antibody is a powerful new tool for systems that are important in several biological processes, as the market sales of the anti-depressant drug fluoxetine demonstrate. It is expected, and our preliminary data are confirming, that this antibody will also be excellent for *in vivo* targeting of SERT-positive neurons.

Figure 2. Immunostaining of the rat raphe nucleus with the anti-SERT monoclonal antibody and anti-Cy5 second antibody.

Photo provided by Dr. George Richerson



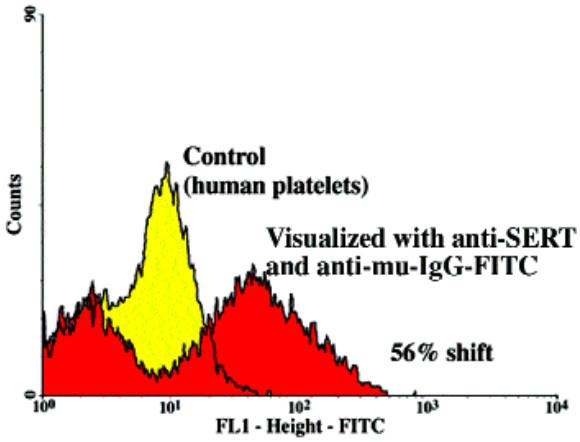


Figure 1.
SERT from human platelets is 92% identical to the rat protein and the antigenic peptide selected is identical in both humans and rats. Anti-SERT binds SERT on human platelets, showing a moderate to high level of expression. The platelets were incubated with anti-SERT at a 1:50 dilution, and a Pharmingen anti-mu-IgG-FITC conjugate at a 1:50 dilution. Platelets were run on a FACScan (Becton-Dickinson) and the data analyzed using Lysis II.

Coming Soon!

SERT-SAP
Targeted Toxin

New Neurotransmitter Antibodies

L-Glutamine (AB-T13)		NO-L-Glutamine (AB-T14)	
Species Reactivity:	all species	*Species Reactivity:*	all species
Description:	Polyclonal antisera were raised in rats after immunization with the conjugates: L-Glutamine-Glutaraldehyde-Carriers.	*Description:*	Polyclonal antisera were raised in rats after immunization with the conjugates: NO-L-Glutamine-Glutaraldehyde-Carriers.
Dilution:	The anti-conjugated L-Glutamine antibody can be diluted between 1:2,000-1:5,000.	*Dilution:*	The anti-conjugated NO-L-Glutamine antibody can be diluted between 1:2,000-1:5,000.

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.

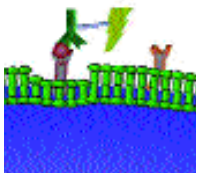
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

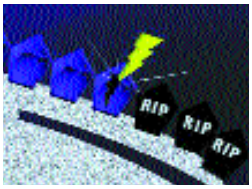
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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

STRESENOIS

JOINTCENI

NORSETINO

TOPPAHORGH

DROPEW

WHAT TO DO WHEN RESULTS SEEM FISHY

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

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, 2002.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

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Targeting Trends

Reporting the latest news in Molecular Surgery

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Newsletter Highlights

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

HCRT-SAP Lesion Produces Sleepiness While anti-DBH-SAP Lesion Does Not

Carlos Blanco-Centurion contributes this report resulting from work done for an abstract at the 2001 Society for Neuroscience meeting: ***Hypocretin B-Saporin Lesions of the Brainstem Increase REM Sleep at Night*** *CA Blanco-Centurion, RJ Salin-Pascual, D Gerashchenko, MA Greco, PJ Shiromani. Harvard Medical School & UNAM, Mexico City.* This abstract won the ATS Abstract Award. Note: HCRT-SAP is also known as orexin-SAP.

Loss of hypocretin (HCRT, orexin) neurons has been linked to narcolepsy.[1] These neurons project widely throughout the brain,[2] but it is not known which projection to which target site produces what symptom of narcolepsy. Recently we showed that HCRT receptors are present in brainstem areas implicated in REM sleep.[3] Since abnormal triggering of REM sleep characterizes narcolepsy, we have used HCRT-SAP, a targeted toxin that selectively lesions HCRT-2 receptor-bearing cells, to assess the effect on sleep. We also used anti-DBH-SAP to destroy specifically noradrenergic neurons of the locus coeruleus (NA-LC), which are the major brainstem output of HCRT neurons. We knew that hypocretin neurons project to the pons but the goal of this study was to determine which pontine neurons regulate sleep.

Methodology. Rats were implanted with electrodes for polysomnographic recordings. At the same time rats received either a bilateral single microinjection of pyrogen-free

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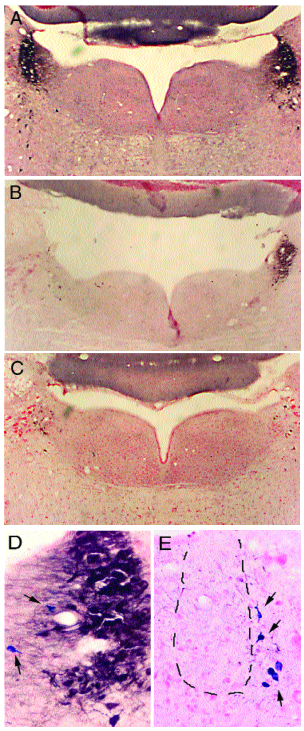


Figure 1. Panel A=saline, B=unilateral lesion with HCRT-SAP, C=bilateral lesions with HCRT-SAP. D and E are magnifications showing the effect of saline and anti-DBH-SAPmicroinjections respectively. Arrows indicate NADPH+ cells (cholinergic).

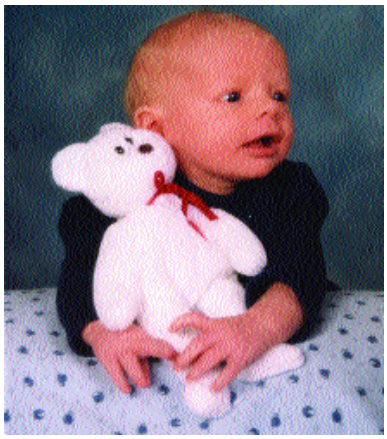
SP-SAP licensed to Cell Targeting Technologies

Advanced Targeting Systems has exclusively licensed the Substance P-Saporin (SP-SAP) compound to Cell Targeting Technologies for development as a pharmaceutical agent. ATS and collaborators at University of Minnesota and University of California, San Diego have completed several preclinical studies using SP-SAP and have thus far found it safe and effective in two animal models. The journal *Science* reported the results of studies in a rat chronic pain model showing that SP-SAP appears to eliminate chronic pain permanently while not interfering with the perception of normal, acute pain sensations. Cell Targeting Technologies will raise capital to fund in-depth toxicology studies and plans to bring SP-SAP to clinical trial within the next two years.

Matthew Kohls Welcomes the Diapered Duo

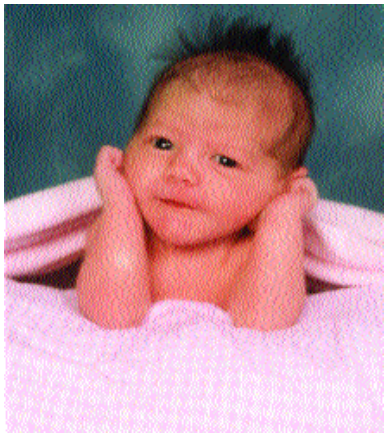
The Kohls welcomed Samuel Jeffrey and Malia Allison into the world on Valentine's Day, February 14, 2002. Sam and Malia continue the family tradition of holiday birthdays; big sister, Sierra Ashlyn, was born on the Fourth of July, 1994. The twins are growing, smiling and ready to rule the world!

Matt is back in the lab here at ATS after taking some time off to lend a hand at home and bond with the babies. Angela (proud mother) has recovered from the birth and is more than thrilled with her two new bundles of joy. Dad, Mom, Sierra and babies are all healthy and happy, if a little short of sleep.



Samuel Jeffrey Kohls
7 lb 11 oz 20 1/2 in

The Kohls family (from left to right): Matthew, Sam, Sierra, Malia, and Angela.



Malia Allison Kohls
8 lb 19 1/4 in

Upcoming Events

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

Experimental Biology
New Orleans, LA • April 20-24
Booth #238

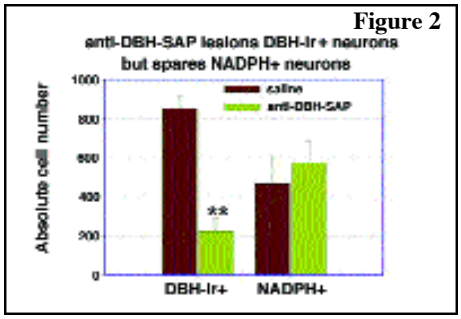
HCRT-SAP Not ANTI-DBH-SAP Produces Sleepiness

(continued from page 1)

saline solution or anti-DBH-SAP (1 mg/mL; vol=80 nL) aimed to the LC nuclei. Afterward, polysomnographic recordings were done across 24 h on the 3rd, 6th, 9th, 12th and 18th days post-injection (12:12h lights on/off). Scoring was made visually on a computer (Icelus software) in 12s epochs for waking, slow wave sleep (SWS) and REM sleep by one technician blind to treatment. ANOVA and t-test with Bonferroni correction (where appropriate) were used to compare changes in sleep parameters. After recordings were done, subjects were sacrificed, brains fixed, removed and sectioned for immunohistochemistry staining for DBH (1:50K). Histochemistry for NADPH was made as well. A blind-treatment technician counted DBH-ir and NADPH+ cells across mesopontine tegmentum in a 1:5 sections protocol.

The experimental protocol was similar to anti-DBH-SAP lesions for HCRT-SAP (100 ng/mL), except it was microinjected lateral to LC and in larger volume (500 nL). In addition to DBH and NADPH labeling, brain sections were immunostained for the specific nuclear protein NeuN to outline the cell loss area. In all cases avidin-biotin-DAB method was used to visualize the antigens.

Figure 2



Results. Post mortem analysis revealed anti-DBH-SAP destroyed noradrenergic neurons in the LC but spared NADPH+ cells (Figs. 1,2). The anti-DBH-SAP pontine lesions did not affect sleep either during day or night period (Fig. 3). This suggests noradrenergic LC neurons are not important in maintaining wakefulness. In contrast, HCRT-SAP injections into the dorsolateral pons increased significantly both SWS and

(continued on page 6)

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

GABAergic septohippocampal neurons are not necessary for spatial memory

Pang KCH, Nocera R, Secor AJ, Yoder RM
Hippocampus 11:814-827, 2001.

The medial septum and diagonal band of Broca (MSDB) are necessary for spatial memory. Both cholinergic and GABAergic neuronal populations are present in the MSDB. 192-Saporin (Cat. #IT-01) was used to eliminate cholinergic populations and kainic acid was used to reduce numbers of GABAergic neurons. Both agents were injected (independently or in combination) into the medial septum and each diagonal band of rats (192-Saporin 250 ng MS, 150 ng DB) to determine the importance of GABAergic neurons in the MSDB for spatial memory. The results showed elimination of GABAergic neurons has no impact on spatial memory, while elimination of cholinergic neurons has a mild impact.

Selective immunolesioning of cholinergic neurons in nucleus basalis magnocellularis impairs prepulse inhibition of acoustic startle

Ballmaier M, Casamenti F, Zoli M, Pepeu G, Spano P
Neurosci 108(2):299-305, 2001.

One of the measures for schizophrenia is a deficit in sensorimotor gating (the ability of the brain to filter sensory input to focus on selective stimuli) measured by prepulse inhibition (PPI) of the startle reflex. The authors injected 300 nl of 400 ng/μl 192-Saporin (Cat. #IT-01) into each side of the nucleus basalis magnocellularis (NBM) in rats to examine the effect of NBM cholinergic neuron elimination on the startle reflex. The data show a significant, persistent disruption of the PPI independent of the amplitude of the startle reflex. This suggests the NBM may play an important role in information processing in schizophrenia.

Lack of effect of moderate Purkinje cell loss on working memory

Wrenn CC, Wiley RG
Neurosci 107(3):433-45, 2001.

When 192-Saporin (Cat. #IT-01) is injected intracerebroventricularly, some p75-expressing cerebellar Purkinje cells are eliminated along with cholinergic neurons. To verify that the effects of basal forebrain lesions on working memory were not caused by loss of these Purkinje cells the authors compared doses of 1 μg OX7-SAP (Cat. #IT-02) and either 2 μg or 4 μg of 192-Saporin injected into the lateral ventricle. The data show that although similar amounts of Purkinje cells were eliminated by OX7-SAP and the lower dose of 192-Saporin, no working memory deficits resulted. Only the 4-μg dose of 192-Saporin produced working memory deficits, they conclude that this is not due to Purkinje cell loss, but the loss of cholinergic neurons.

Central cholinergic depletion induced by 192 IgG-saporin alleviates the sedative effects of propofol in rats

Pain L, Jeltsch H, Lehmann O, Lazarus C, Laalou FZ, Cassel JC
Brit J Anaesth 85(6):869-73, 2000.

In order to examine the effect of cholinergic depletion on the sedative potency of propofol in rats the authors injected 1 μg of 192-Saporin (Cat. #IT-01) into each lateral ventricle. The findings indicate a ~50% reduction in sedative potency in lesioned rats.

Transverse patterning reveals a dissociation of simple and configural association learning abilities in rats with 192 IgG-saporin lesions of the nucleus basalis magnocellularis

Butt AE, Bowman TD
Neurobiol Learn Mem 77:211-233, 2002.

Using 80 ng bilateral infusions of 192-Saporin (Cat. #IT-01) into each of the medial and lateral target sites of the nucleus basalis magnocellularis (NBM) in rats, the authors demonstrate that lesioning the cholinergic systems of the NBM impairs a more complicated learning technique, while leaving simple association learning intact. The results also show that the transition between these two learning strategies is impaired in lesioned animals.

(continued on page 4)

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

Targeting Topics: Recent Scientific References

(continued from page 3)

Facilitation of dopamine-mediated locomotor activity in adult rats following cholinergic denervation

Mattsson A, Ögren SO, Olson L
Exp Neurology 174:96-108, 2002.

It has been hypothesized that hyperactivity of dopaminergic systems is involved in schizophrenia. The authors examine the effect of central cholinergic denervation on dopamine-mediated functions. Adult rats received bilateral injections of 2.5 μg of 192-Saporin (Cat. #IT-01) into the lateral ventricles. Neonatal rats received 400 ng 192-Saporin in bilateral i.c.v. injections. The results indicate that severe cholinergic deficiencies in the forebrain can lead to dopaminergic overfunction, one of the possible mechanisms in schizophrenia.

Isoflurane and nociception: Spinal alpha2A adrenoceptors mediate antinociception while supraspinal alpha1 adrenoceptors mediate pronociception.

Kingery WS, Agashe GS, Guo TZ, Sawamura S, Davies MF, Clark JD, Kobilka BK, Maze M
Anesthesiol 96:367-74, 2002.

The authors injected 3 μg/3 μl of anti-DBH-SAP (Cat. #IT-03) into the lateral ventricle of rats to determine whether noradrenergic brainstem nuclei and descending spinal pathways are responsible for the antinociceptive actions of isoflurane. The results indicate that isoflurane modulates nociception by as many as three mechanisms, utilizing various combinations of noradrenergic neurons, adrenoceptors, and descending spinal pathways.

Cytotoxic targeting of isolectin IB4-binding sensory neurons

Vulchanova L, Olson TH, Stone LS, Riedl MS, Elde R, Honda CN.
Neurosci 108(1):143-55, 2001.

Vulchanova *et al*. examine the role of IB4-binding neurons in nociception. IB4-SAP (Cat. #IT-10) was injected into rats (2 μg in left sciatic nerve). The resulting ablation of IB4-binding neurons provides evidence for their role in nociceptive processing and demonstrates a rapid compensatory response to signalling of acute pain.

Lesions of the nucleus basalis magnocellularis induced by 192 IgG-saporin block memory enhancement with posttraining norepinephrine in the basolateral amygdala

Power AE, Thal LJ, McGaugh JL
PNAS 99(4):2315-2319, 2002.

There is evidence that memory consolidation (retention) can be modulated by drugs and stress hormones acting in the basolateral amygdala (BLA). The BLA sends projections to the nucleus basalis magnocellularis (NBM), which in turn sends cholinergic projections to the neocortex. The authors used 100 ng bilateral infusions of 192-Saporin (Cat. #IT-01) in 500 nl 0.1 M PBS to investigate whether lesions of the cholinergic NBM projections affect BLA modulation of memory. 192-Saporin lesions blocked memory enhancement normally induced by norepinephrine infusions into the BLA. This finding suggests NBM-cortex projections may mediate BLA modulation of memory storage or processing in the neocortex.

Cholinergic and noncholinergic septal neurons modulate strategy selection in spatial learning

Cahill JFX, Baxter MG
Eur J Neurosci 14:1856-1864, 2001.

The authors compared ibotenic acid (IA)-treated rats with those injected with 45 ng and 30 ng of 192-Saporin (Cat. #IT-01) into two separate coordinates of the medial septum/vertical limb of the diagonal band (MS/VDB) to investigate the role of basal forebrain projections in modulating strategy selection in spatial learning. While rats with IA lesions in the MS/VDB demonstrated significant disruption of the learning process, the 192-Saporin-lesioned rats did not show this effect.

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

Targeting Talk: *Toxin Safety*

by Dr. Douglas A. Lappi

Q: Your recent issue of Targeting Trends stated that it was unlikely that saporin compounds or constituents would be excreted in urine or feces. However, you acknowledge that experimental data is lacking. Have there been any tests of animal urine or feces for saporin content? My animal care staff are concerned.

A: One of the reasons that no studies have been done on excretion of saporin is that there isn't much on the theoretical side to cause concern. The primary issue is that the quantity used in mice (and even rabbits) is so small that when looked at in human terms (i.e., an animal 10 to 100-times larger), the dosage becomes insignificant. The LD_{50} for saporin in mice is 4-8 mg/kg;[1] that would translate in humans to more than you'll ever use! The immunotoxins, which contain only about 20% saporin by weight, really do not contain all that much saporin.

Looking at it another way, you need a concentration of about 100 nM to see even a vague hint of toxicity of saporin to cells. In human blood, that would correspond to 24 mg injected systemically into a person. It would be really expensive for anyone to get close to that number.

As far as urine and feces goes, the same calculations are appropriate, but there will be considerable degradation – the protein content in urine and feces is quite low and the probability is that you will be dealing with only saporin. Remember saporin is a plant protein that is related to proteins in foods that we eat (cucumbers, for example).

Reference

1. Stirpe F, Derenzini M, Barbieri L, Farabegoli F, Brown AN, Knowles PP, Thorpe PE (1987) Hepatotoxicity of immunotoxins made with saporin, a ribosome-inactivating protein from *Saponaria officinalis*. *Virchows Arch [B]* 53:259-271.

Q: Are there any studies which indicate what doses of saporin (by itself or compounded with an antibody) would be hazardous if ingested or injected (i.e. systemic dose level resulting in death or organ dysfunction).

A: When there is an antibody that does recognize a human epitope (the human p75-saporin immunotoxin that is used in rabbits, for example), at about 1 pM one sees the slightest bit of toxicity to cells. That translates, if injected by error into a human blood supply, to about 170 micrograms. That also is a gigantic dose. I am using very conservative numbers here, and the bottom line is that you cannot accidentally reach such dangerous levels under normal handling situations.

Having said all this, we still recommend that our customers take excellent care of themselves and we state clearly that precautions should be taken by people handling these materials, just as they should use precautions with all laboratory chemicals. Please refer to the data sheets provided with our products for safety instructions.

Saporin (Cat. # PR-01)
a ribosome-inactivating protein from seeds of the plant
Saponaria officinalis

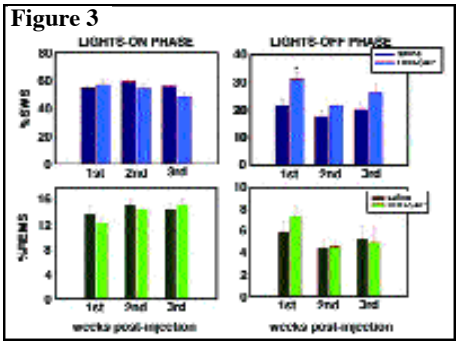
Saporin is effective as a control in experiments with targeted saporin immunotoxins or ligand toxins.

Also available:
Anti-Saporin (Goat) AB-15
Anti-Saporin (Chicken, affinity purified) AB-17AP

HCRT-SAP Not ANTI-DBH-SAP Produces Sleepiness

(continued from page 2)

Figure 3



REM sleep. This effect was only present during the normal rat active period (night), but not so during the rest period. The sleepiness induced by HCRT-SAP pontine lesions is mainly generated by an increase of the sleep drive, that is, an increase in the number of episodes at night ($p<0.05$). Sleepiness was also associated with a significant reduction in the number of NeuN-ir within the parabrachial complex as well as NADPH+ cells in the LDTg (laterodorsal tegmental). Yet HCRT-SAP did not affect DBH-ir (Figs. 4, 5). Our results suggest both parabrachial and LDTg cholinergic neurons, but not noradrenergic LC neurons, express the hypocretin-2 (orexin B) receptor; anti-DBH-SAP only affects noradrenergic LC neurons. Additionally these HCRT-SAP sensitive neurons in the dorsolateral pons seem to be involved in sustaining waking during the normal active period. As a result, the daytime sleepiness observed in narcoleptic HCRT-deficient subjects could be associated with a lack of excitatory input to parabrachial and LDTg rather than to LC neurons, as has been proposed.

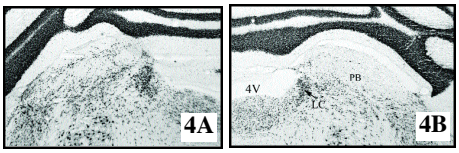
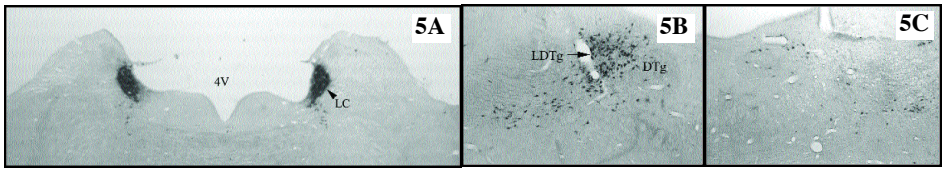


Figure 4. Panel A=saline, Panel B=HCRT-saporin (100 ng/μl, TV=500 nl). Both sections (50 μm=thickness) were immunostained to label the specific neuron's nuclear protein NeuN. Magnification in both cases is 4X. 4V=Fourth ventricle, LC=Locus coeruleus, PB=Parabrachial nuclei.

References

1. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E (2000) *Lancet* 355:39-40.
2. Peyron C, Tighe DK, Van de Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS (1998) *J Neurosci* 18:9996-10015.
3. Greco MA, Shiromani PJ (2001) *Mol Brain Res* 88:176-182.
4. Gerashchenko D, Kohls MD, Greco M, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ (2001) *J Neurosci* 21:7273-7283.

Figure 5. HCRT-SAP(100 ng/μl; vol=500 nl) spares DBH-IR cells in the LC (A) but destroys NADPH+ cells (cholinergic) in the LDTg (C). (B=saline; C=HCRT-SAP).



Targeting Ticklers

A Brief Guide to Scientific Literature

The following phrases, frequently found in technical writings, are defined below for your enlightenment.

Phrase	Translation
It has been long known	I haven't bothered to check the references
It is known	I believe
It is believed	I think
It is generally believed	My colleagues and I think
There has been some discussion	Nobody agrees with me
It can be shown	Take my word for it
It is proven	It agrees with something mathematical
Of great theoretical importance	I find it interesting
Of great practical importance	This justifies my employment
Of great historical importance	This ought to make me famous
Some samples were chosen for study	The others didn't make sense
Typical results are shown	The best results are shown
Correct within order of magnitude	Wrong
The values were obtained empirically	The values were obtained by accident
The results are inconclusive	The results seem to disprove my hypothesis
Additional work is required	Someone else can work out the details
It might be argued that	I have a good answer to this objection
The investigations proved rewarding	My grant has been renewed

Targeting Teaser Winners

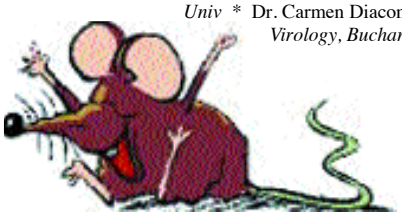
Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

The solution to the puzzle was:

Jumbles: RESTENOSIS INJECTION SEROTONIN PHOTOGRAPH POWDER

Answer: What to do when the results seem fishy --- WEIGHTHEOPTIONS

WINNERS: April Henderson, *UC Irvine* * Randy Willis, *SkyePharma* * Douglas J Taatjes, *Univ of Vermont* * Christopher Flores, *UTHealth Sci Ctr, San Antonio* * Dr. David G Watson, *Univ North Texas Health Sci Ctr* * Chuck Sevigny, *Univ Virginia Health System* * Dr. George M Anderson, *Yale Univ* * Dr. Robert Speth, *Washington State Univ* * Dr. Carmen Diaconu, *Inst of Virology, Bucharest, Romania*



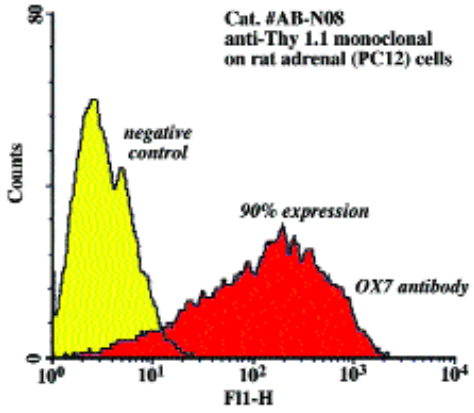
Targeting Tools: Featured Products

OX7-SAP

OX7-SAP is a cytotoxin to many types of neurons. It is one of the most potent of the saporin immunotoxins—the conjugates between a cell surface antibody and the ribosome-inactivating protein from *Saponaria officinalis*. The reason for the potency could be due to the construct's grand tradition. The antibody, OX7, is one of the original antibodies to cell surface proteins produced by England's Medical Research Council, the famous MRC of Crick, Perutz, Brenner and many others. This antibody was made by injection of rat white blood cells for immunization and recognizes CD90 or Thy 1. Thy 1 is expressed on virtually all neurons after cessation of axonal growth.[1] The mouse monoclonal recognizes rat and mouse Thy 1. Figure 1 illustrates analysis by flow cytometry of PC12 cells with OX7 and demonstrates a remarkable shift.

To the neuroscience community, OX7-SAP has a distinguished career. It was used by Ron Wiley and his colleagues in the first applications of immunotoxins in the nervous systems. He used it as a "suicide transport" agent, in which the injection is at a terminal site with the purpose of eliminating the cell that projects to that point through uptake at the terminal, transport to the cell body and eventual death by protein synthesis inhibition.[2] He also used OX7-SAP by intraventricular injection for the subsequent removal of Purkinje cells.[3] OX7-SAP has been used by numerous investigators in variations on this theme. The immunotoxin has also been used to deplete mouse blood of T lymphocytes, because these also express Thy 1.[4] (See the ATS website for a complete list of references.)

Figure 1



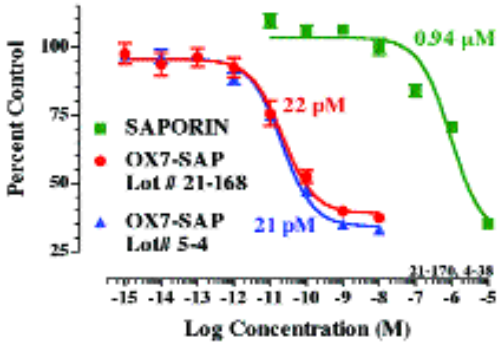


Figure 2. Cytotoxicity of two different lots of OX7-SAPand of non-conjugated saporin (SAP) to PC12 cells in culture. Cells were plated at 5000 cells per well and allowed to acclimate. Samples were added at the indicated concentrations and cells were incubated for 72 hours. MTS (Promega) was added and, after color development, wells were read with a Molecular Dynamics SpectraMax 340. ED_{50}'s of each compound are color-coded. Data analysis is by PRISM by GraphPad.

Figure 2 shows the cytotoxicity of OX7-SAP to PC12 cells in culture. There is a greater than 4 orders of magnitude difference between the non-conjugated saporin and the OX7-SAP which is targeted to PC12 cells because of their Thy 1 expression.

For an excellent discussion on the many applications for OX7-SAP in lesioning of neurons, see the recent review by Wiley and Kline.[5]

References

1. Tiveron MC, Barboni E, Pliego Rivero FB, Gormley AM, Seeley PJ, Grosveld F, Morris R, Berger-Sweeney J, Stearns NA, Murg SL, *et al*. (1992) Selective inhibition of neurite outgrowth on mature astrocytes by Thy-1 glycoprotein. *Nature* 355:745-748.
2. 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.
3. Davis TL, Wiley RG (1989) Anti-Thy-1 immunotoxin, OX7-saporin, destroys cerebellar Purkinje cells after intraventricular injection in rats. *Brain Res* 504:216-222.
4. Marcucci F, Lappi DA, Ghislieri M, Martineau D, Siena S, Bregni M, Soria M, Gianni AM (1989) *In vivo* effects in mice of an anti-T cell immunotoxin. *J Immunol* 142:2955-2960.
5. Wiley RG, Kline IR (2000) Neuronal lesioning with axonally transported toxins. *J Neurosci Meth* 103:73-82.

Featured Antibody

AB-N08 Antibody to OX7 (anti-Thy 1)

Species Reactivity:	Rat and Mouse Thy 1.1
Applications:	Immunohistochemistry; immunostaining; immunofluorescence (cells); radioimmunoassay; causes glomerulosclerosis when injected intravenously in rats and mice
Reference:	Narita *et al*. (1997) *Kidney Int Suppl* 63:S215-217.

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.

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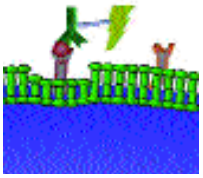
[§]or growth factor, peptide, ligand, or cytokine

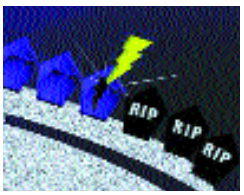
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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.

HOGPHUTTUR

PLOMMYAH

CLEENORT

STINGECIET

SLAIDYSI

WHY THE SCIENTIST WAS EXCITED TO HEAR HIS AGRICULTURE GRANT WAS FUNDED

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

Answer:

IT WOULD... ○○○○ ○○○ ○○○ "○○○○○"

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Inside this issue:

Newsletter Highlights

- Featured Antibodies *(page 3 and page 4)*
- Neuropeptide Toxins *(page 5)*
- Anti-Tac and Anti-Tac-SAP *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Saporin Immunotoxins for Treating Human Hematological Malignancies

Contributed by David J Flavell, PhD, The Simon Flavell Leukaemia Research Unit, Division of Cancer Sciences, University of Southampton Medical School, United Kingdom.

I have asked Dr. Flavell to contribute an article on the clinical use of his saporin immunotoxins. Dr. Flavell's research unit is named after his son, who died from leukemia. At that point, he and his wife, Bee, dedicated their lives to finding a cure for childhood cancers. You are encouraged to contribute to Dr. Flavell's efforts that are more fully described at the following website:

www.leukaemiabusters.org.uk.

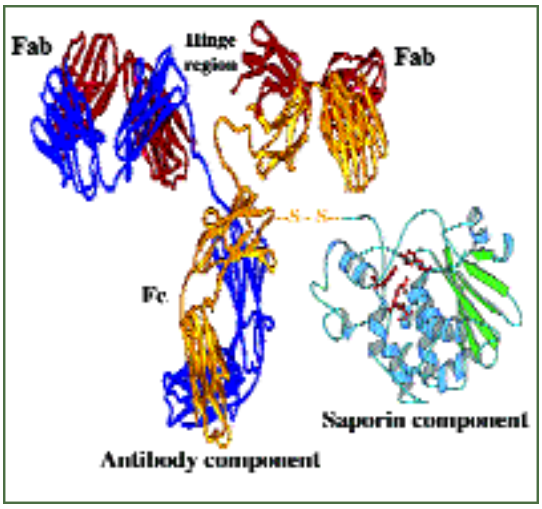
Douglas A. Lappi,
President/Chief Scientific Officer

Immunotoxins have a clinical utility in any human condition where the elimination of an unwanted cell population is desired. The most obvious candidate disease is cancer and consequently the vast majority of early phase clinical studies with immunotoxins have centered on the treatment of human malignancies with the goal of eliminating cancer cells from the patient's body. Most of these clinical trials have been conducted using ricin A chain or blocked ricin[1] as the toxin component. Whilst there have been anti-tumor responses with ricin-based immunotoxins, particularly in hematological malignancies,[2] their toxicity profile has limited their clinical usefulness and interest in their utility has consequently dropped away. In contrast, there have been very few clinical studies with saporin-based immunotoxins (Fig 1). The earliest study was conducted by Falini and his associates[3] in Italy and Germany utilizing a CD30 antibody coupled to saporin for the treatment of four patients with advanced Hodgkin's disease. Two patients showed dramatic responses to treatment and no serious side effects were observed. In particular, vascular leak syndrome, a potentially fatal toxicity that had been associated with ricin-based immunotoxins, was not observed.

These initial results encouraged us to undertake preclinical investigations with immunotoxins directed against the B-cell restricted CD19 antigen for B-lymphoid tumors and against the CD38 molecule for multiple myeloma. The development of such therapeutic molecules required robust preclinical investigations to determine their toxicity profiles in animals, the normal

(continued on page 2)

Figure 1. Idealized molecular ribbon diagram of the appearance of an immunotoxin with saporin coupled covalently via a disulfide bond to a mouse monoclonal antibody.

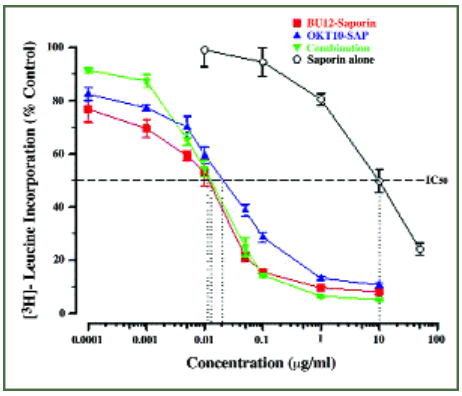


Saporin Immunotoxins for Treating Human Hematological Malignancies

(continued from page 1)

tissue-binding specificities, and to demonstrate their selective anti-tumor activity both *in vitro* and *in vivo*. Such preclinical investigations are mandatory to justify taking an experimental drug into patients and to ensure, as far as possible, safety of the drug. A poignant reminder here was the disastrous peripheral nerve damage experienced by women with breast cancer treated with a ricin A chain-based immunotoxin which unexpectedly also targeted nervous tissue.[4] The most sensitive *in vitro* method for determining the selective cytotoxic potency of saporin-based immunotoxins is by measuring their ability to selectively inhibit protein synthesis in antigen-expressing cell lines in a dose-dependent manner. This is clearly shown in Figure 2, where increasing concentrations of two immunotoxins: anti-CD19 (BU12-Saporin) and anti-CD38 (OKT10-SAP) show decreasing

Figure 2 Protein synthesis inhibition in the CD19+/CD38+ acute lymphoblastic cell line, NALM-6, following 48h exposure to increasing concentrations of BU12-Saporin, OKT10-SAP, a combination of both immunotoxins, or saporin alone. The IC_{50} is shown as the point on the x-axis intercept representing the concentration of ITthat inhibits protein synthesis in the target cell line by 50% relative to untreated control cells. Note that the use of a combination of both ITs does not result in any increased cytotoxicity for the target cell line in this particular assay.



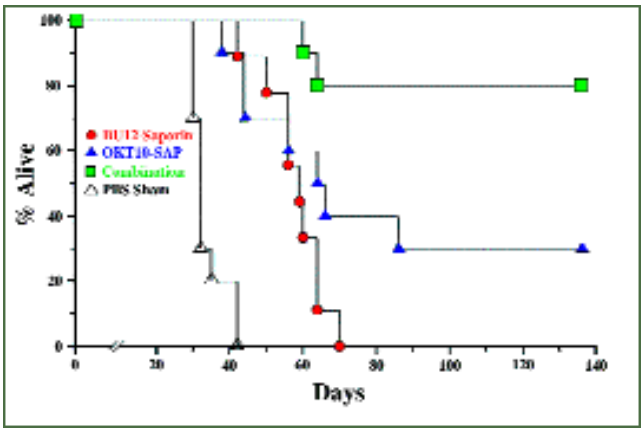


Figure 3. Survival curves of SCID mice xenografted with the human ALLcell line NALM-6 and treated i.v. seven days later with three 10-µg doses of BU12-Saporin, OKT10-SAP, a combination of both ITs (5 µg of each) or sham treated with PBS. Note that greater numbers of SCID-NALM-6 mice survive significantly longer following treatment with the IT combination.

protein synthesis levels in a target CD19+ CD38+ pediatric acute lymphoblastic leukaemia (ALL) cell line. Note that when the two immunotoxins were used in combination no improvement in performance was seen. When the same two immunotoxins were used individually *in vivo* in SCID mice xenografted with the same ALL cell line, they exerted therapeutic activity in line with the data obtained from the *in vitro* study. However, when the two immunotoxins were used in combination the therapeutic effect was significantly better than either individual drug used alone (Fig 3). This *in vivo* result could not have been predicted from the short-term protein synthesis inhibition assay. We have also made similar findings with *in vivo* models of human B-cell lymphoma[5] and T-cell leukaemia.[6] These observations have provided us with the longer-term goal of conducting clinical trials with combinations of different immunotoxins.

Following preclinical evaluation my laboratory is now directly involved in two separate Phase I clinical trials with BU12-Saporin and OKT10-SAP currently underway in the United Kingdom. Working together with the United Kingdom Children's Cancer Study Group and Cancer Research UK, we are now treating children with relapsed ALL with BU12-Saporin at 11 children's cancer units and adult myeloma patients with OKT10-SAP at two centers. These are early phase dose escalation studies whose primary aims are to determine the maximum tolerated dose (MTD) and dose limiting toxicities (DLT) for both saporin immunoconjugates for use in subsequent Phase II studies. The secondary objective of these studies is to define the pharmacokinetic profile of these drugs in patients and to document any tumor responses.

In conventional Phase I studies, cohorts of three patients receive a fixed dose of drug and are then monitored for toxic side effects, which are carefully graded according to strictly laid down criteria. If toxic side effects occur below grade 3, then the next cohort of patients is permitted to receive the next dose

(continued on page 6)

Upcoming Events

3rd Forum of European Neuroscience (FENS)
Paris France • July 13-17, 2002 • Booth #229

Society for Neuroscience (SFN)
Orlando Florida • November 3-6, 2002

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Impairments in negative patterning, but not simple discrimination learning, in rats with 192 IgG-Saporin lesions of the nucleus basalis magnocellularis

Butt AE, Noble MM, Rogers JL, Rea TE
Behav Neurosci 116(2): 241-255, 2002

192-Saporin (Cat. #IT-01) administration to the basal forebrain has frequently been used in rats to create a model for Alzheimer's disease. The authors used 0.2 μl bilateral injections of 0.4 μg/μl 192-SAP into the nucleus basalis magnocellularis (NBM). Previous studies using non-specific excitotoxic agents have suggested the involvement of the NBM in learning and memory. The authors confirm more recent findings that indicate some of the deficits produced by these excitotoxins are due to the non-specific lesioning caused by these agents. The highly selective cholinergic lesioning produced by 192-Saporin left simple association learning intact but impaired more complicated configural association processes.

Featured Antibody

AB-N10 Antibody to Substance P

Substance P (SP) is an undecapeptide belonging to the tachykinin peptide family. It is widely distributed throughout the central and peripheral nervous systems and has been implicated as having a role in the transmission of pain.

Specificity:	Substance P
Applications:	ELISA and western blot

Visit the ATS website for a complete list of products and references.

Alpha-7 nicotinic receptor expression by two distinct cell types in the dorsal raphe nucleus and locus coeruleus of rat

Bitner RS, Nikkel AL
Brain Res 938: 45-54, 2002

Neuronal nicotinic acetylcholine receptors (nAChRs) are suspected to play a role in neurophysiological disorders such as schizophrenia, Alzheimer's disease, and epilepsy. Whereas the molecular and cellular properties of these receptors have been well characterized, the role of nAChRs in the nervous system is as yet unclear. The authors injected rats intracerebroventricularly with 5 μg/5 μl of anti-DBH-SAP (Cat. #IT-03) to eliminate the noradrenergic nuclei. Using these data along with data acquired by elimination of serotonergic nuclei with 5,7-DHT, the authors showed that both noradrenergic nuclei in the locus coeruleus and serotonergic nuclei in the dorsal raphe nucleus express the alpha-7 nAChR subunit.

Depressor and tachypneic responses to chemical stimulation of the ventral respiratory group are reduced by ablation of neurokinin-1 receptor-expressing neurons

Wang H, Germanson TP, Guyenet PG
J Neurosci 22(9): 3755-3764, 2002

The pre-Bötzinger complex is a region of the ventral respiratory group (VRG) in the brain. Injection of excitatory amino acids into this region can cause a variety of responses such as rapid breathing, hypotension, and elevated arterial pressure. The authors used SSP-SAP (Cat. #IT-11) to eliminate the neurokinin-1 receptor (NK-1r) positive neurons in the VRG to determine their role in control of respiration and arterial pressure. Intraparenchymal injection of 0.313 ng/50 nl SSP-SAP produced several abnormal respiratory effects in rats treated with excitatory amino acids. The results indicate that NK-1r positive neurons in the ventrolateral medulla play an important role in respiratory rhythm and blood pressure.

Interactions between aging and cortical cholinergic deafferentation on attention

Burk JA, Herzog CD, Porter MC, Sarter M
Neurobiol Aging 23: 467-477, 2002

Trauma to forebrain cholinergic neurons is suspected to make these neurons more susceptible to future age-related loss of function. The authors tested this theory by making incomplete lesions of the basal forebrain cholinergic system using bilateral infusions of 192-Saporin (0.5 μl of 0.15 μg/μl, Cat. #IT-01) in rats trained prior to surgery. The attentional performance of the treated rats did not differ from control animals until the age of 31 months. The data indicate that pre-existing damage to the cholinergic basal forebrain region yields age-related attentional impairments.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of 192 IgG-saporin on attentional performance in a five-choice serial reaction time task

McGaughy J, Dalley JW, Morrison CH, Everitt BJ, Robbins TW
J Neurosci 22(5):1905-1913, 2002

192-Saporin (Cat. #IT-01) has been a very useful tool in determining the role of the basal forebrain cholinergic system in arousal and attention tasks. The authors lesioned the nucleus basalis magnocellularis of rats with an infusion of 0.5 μl per hemisphere of 0.15 μg/μl or 0.45 μg/μl 192-Saporin. The data show a correlation between the extent of the lesion and the amount of impairment in an attentional task. The authors also found that the accuracy deficits in the task could be ameliorated by lengthening the stimulus time, or exacerbated by increasing the event rate. Taken together the data indicate a direct relationship between basal forebrain damage and impaired attentional function.

Septal grafts and evoked acetylcholine release in the rat hippocampus after 192 IgG-saporin lesions

Birthelmer A, Dommes E, Jeltsch H, Cassel JC, Jackisch R
Regen Transplant 13(7):973-976, 2002

The authors investigate the structural and behavioral effects of intrahippocampal grafts containing cholinergic neurons into a lesioned region of the brain. Previous studies in rats were complicated by the lack of a specific cholinergic lesioning agent. 0.4 μg 192-Saporin (Cat. #IT-01) in 0.4 μl was injected into the vertical limb of the diagonal band of Broca in rats, then 6 to 10 months later the animals received intrahippocampal grafts of septal cells containing cholinergic neurons. Measurement of noradrenaline and serotonin uptake indicate that the grafts were able to produce only modest cholinergic effects. The authors conclude that this may be a result of performing the graft too soon following administration of the immunotoxin.

Time-dependent descending facilitation from the rostral ventromedial medulla maintains, but does not initiate, neuropathic pain

Burgess SE, Gardell LR, Ossipov MH, Malan Jr TP, Vanderah TW, Lai J, Porreca F
J Neurosci 22(12):5129-5136, 2002

Various indications, such as declining afferent discharge over time, suggest that the mechanisms involved in persistent neuropathic pain are different than those that initiate the pain. The authors have previously shown that cells expressing the mu-opioid receptor are involved in the descending pain pathway. In this work, the authors lesioned the rostral ventromedial medulla (RVM) in rats using 1.5 pmol in 0.5 μl of dermorphin-SAP (Cat. #IT-12) administered to each side of the RVM. Measurements of pain-related behavior show that mu-opioid receptor-expressing cells in the RVM are involved in the maintenance of heightened sensitivity to stimuli seen in neuropathic pain.

NEW!

Featured Antibody

AB-N11 Antibody to VAChT

VAChT (Vesicular Acetylcholine Transporter) is expressed selectively in all known cholinergic neurons. Antibodies to VAChT are useful in visualizing acetylcholine (ACh)-containing neurons. After release of ACh from the synaptic vesicles, transmission is stopped when ACh is broken down in the synaptic clefts by acetylcholinesterase. The choline formed from this breakdown is recycled by the high-affinity choline transporter.

Species Reactivity:	Rat
Applications:	FACS analysis

Visit the ATS website for a complete list of products and references.

Targeting Talk: *Neuropeptide Toxins*

by Dr. Ronald G. Wiley

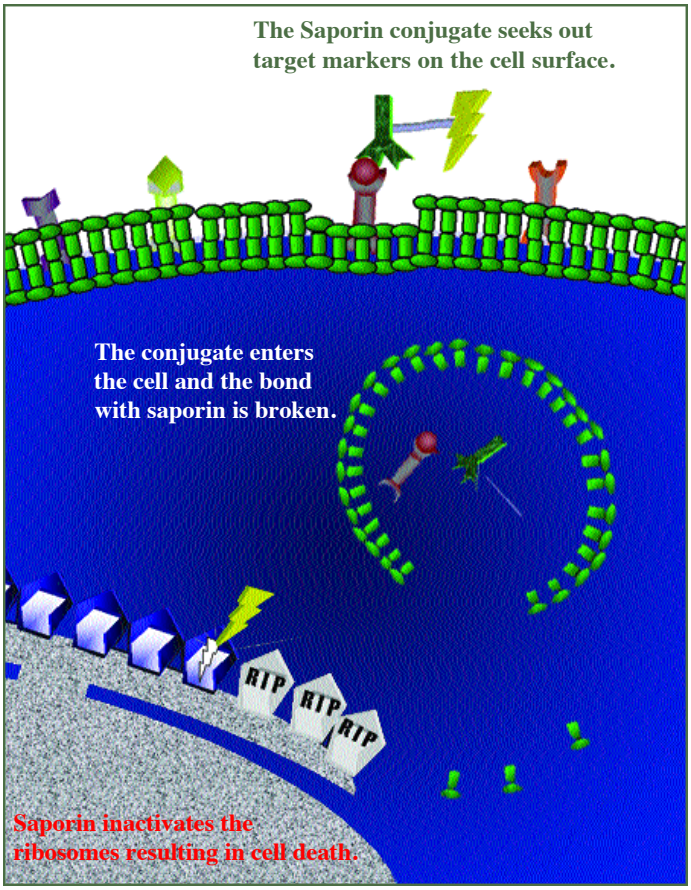
Q: What are neuropeptide-toxins and how do they work?

A: Neuropeptide-toxin conjugates are made up of the ribosome-inactivating protein, saporin, coupled to a naturally-occurring or synthetically-modified neuropeptide such as Substance P or dermorphin. The conjugate has binding specificity similar to the native, unconjugated neuropeptide.

When the neuropeptide binds to its cognate receptor, the conjugate is internalized. Once inside the target cell within an endosome, the neuropeptide and saporin separate and some of the saporin translocates into the cytoplasm where it catalytically inactivates ribosomes resulting in cell death.

Q: Are neuropeptide-toxins effective suicide transport agents?

A: The general answer to this question is not currently known. However, in the instance of intrathecally-injected dermorphin-SAP (Cat. #IT-12), the evidence does NOT favor suicide transport of the neuropeptide-toxin conjugate. When supramaximal doses of dermorphin-SAP (750 ng) are injected into the lumbar subarachnoid space of adult rats, less than 1% of lumbar dorsal root ganglion cells show evidence of saporin activity. This is in spite of the fact that many of these neurons express the targeted mu opioid receptor on their central terminals in the superficial dorsal horn of the spinal cord. This assertion is based on analysis of over 16,000 neurons from dorsal root ganglia in six rats.



Dermorphin-SAP (Cat. # IT-12)
a conjugate of the mu opioid receptor (MOR) agonist dermorphin and the ribosome-inactivating protein, saporin

Eliminates mu-opioid receptor-expressing cells

Substance P-SAP (Cat. # IT-07)
a conjugate of Substance P and the ribosome-inactivating protein, saporin

Eliminates Substance P (SP) receptor-bearing neurons

Also available using a more stable analog of Substance P:
SSP-SAP (Cat. #IT-11)

Blank-SAP (Cat. # IT-21)
a conjugate between a non-targeted peptide and the ribosome-inactivating protein, saporin

This molecule is the perfect control for use with peptide conjugates such as: SP-SAP, SSP-SAP, Orexin-SAP, Dermorphin-SAP and CRF-SAP.

Saporin Immunotoxins for Treating Human Hematological Malignancies

(continued from page 2)

level up. The trial continues in this way until the MTD is reached. In our ongoing studies with OKT10-SAP in adult myeloma patients a dose level of 40 μg/kg/day x 5 days has been reached so far without any serious drug-related side effects. Encouragingly, we have not encountered vascular leak syndrome in any patient. Approximately half of the patients have developed human anti-mouse antibodies (HAMA) and a similar number human anti-saporin antibodies (HASA). Pharmacokinetic analysis, though only partially completed and not yet formally analyzed, has revealed some major differences in peak levels and decay rates between different patients. This probably reflects differences in tumor burden between individual patients—it is predicted that patients with higher tumor burdens will achieve lower peak serum levels and a shorter drug half life due to rapid removal of drug from the circulation by the larger antigenic "sink" that a larger tumor burden provides. The BU12-Saporin study in relapsed pediatric ALL is at a less advanced stage and has so far recruited six patients. Once the MTD has been established for both drugs then we can move on to Phase II studies whose primary objective will be to determine the response rate. Then the really exciting work begins!

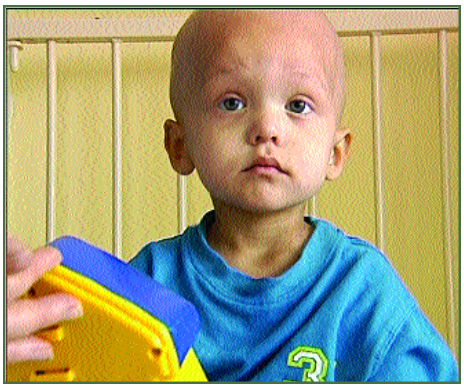


Figure 4. Children with relapsed acute lymphoblastic leukaemia are now being treated at eleven different children's cancer centres around the United Kingdom.

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Targeting Ticklers

Thoughts to Ponder

Why are a wise man and a wise guy opposites?

If horrific means to make horrible, does terrific mean to make terrible?

Why isn't 11 pronounced onety-one?

If a pig loses its voice, is it disgruntled?

If love is blind, why is lingerie so popular?

When someone asks you, "A penny for your thoughts," and you put your two cents in, what happens to the other penny?

Why do we say something is out of whack? What is a "whack"?

Why is the man who invests all your money called a broker?

Why do croutons come in airtight packages? It's just stale bread to begin with.

If you mixed vodka with orange juice and milk of magnesia, would you get a Phillips Screwdriver?

"I am" is reportedly the shortest sentence in the English language. Could it be that "I do" is the longest sentence?

If lawyers are disbarred and clergymen defrocked, doesn't it follow that electricians can be delighted, musicians denoted, cowboys deranged, models deposed, tree surgeons debarked and dry cleaners depressed?

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: THROUGHPUT LYMPHOMA ELECTRON GENETICIST DIALYSIS

Answer: Why the scientist was excited to hear his agriculture grant was funded — IT WOULD HELP HIM GET "AHEAD"

WINNERS: Dr. Chris Flores, Univ Texas Health Science Center, Endontics * Lauren Phillips, Univ Texas Health Science Center, Pharmacology * Dr. Venky Ramakrishna, Argonex Inc * Mark Aitkenhead, UC Irvine, Bio Sci * Dan Binder, Univ Virginia, Psychobiology * Joseph Menonna, E Orange VA Medical Center, Neurology Service * Robert Speth, Washington State Univ, VCAPP

Targeting Tools: Featured Products

Anti-Tac and Anti-Tac-SAP

Advanced Targeting Systems announces the release of two reagents for immunological studies:

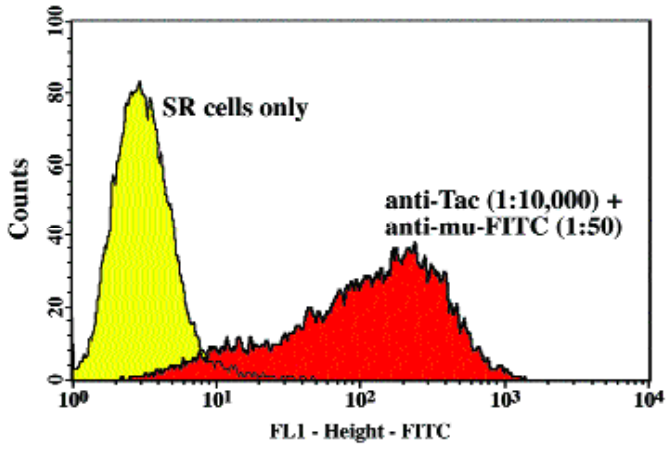
- Anti-Tac (Cat# AB-18) - a monoclonal antibody to human CD25, the interleukin-2 receptor, and
- Anti-Tac-SAP (Cat# IT-23) - the immunotoxin made by conjugation of anti-Tac to saporin.

These reagents add to the growing list of ATS products for use in studies of the hematopoietic systems. Other products include the anti-human and mouse macrophage immunotoxin (Mac-1-SAP, Cat# IT-06) and the anti-rat and mouse T lymphocyte immunotoxin OX7-SAP (Cat# IT-02).

The interleukin 2 receptor (IL-2r) is expressed on activated T lymphocytes and is important for the proliferation of T lymphocytes in response to antigen. The IL-2r is not detected on resting cells.[1] Anti-Tac is able to prevent activation of T lymphocytes by antigen; apparently by binding to a newly available receptor and blocking IL-2 binding.[2] It is reactive with activated and functionally mature human T cells.[3] Figure 1 shows FACS analysis of the antibody bound to SR cells. This cell line is derived from the peripheral blood of a patient with mycosis fungoides. The cells express CD25 and respond to IL-2.

Immunotoxins made with an antibody to the IL-2r have been suggested for clinical use in the treatment of T cell leukemias and lymphomas.[4] We at ATS believe that, as a research tool, this reagent is an important part of the toolbox. Anti-Tac immunotoxins have been shown to remove activated T lymphocytes in culture,[5] and can be used to understand the role of this population in its various processes in the immune system. Figure 2 shows this immunotoxin against SR cells that express CD25. The immunotoxin is more than 100-fold more effective than saporin alone. Later this year we will introduce an anti-rat CD25 immunotoxin that will be useful for *in vivo* studies in animal models.

Figure 1



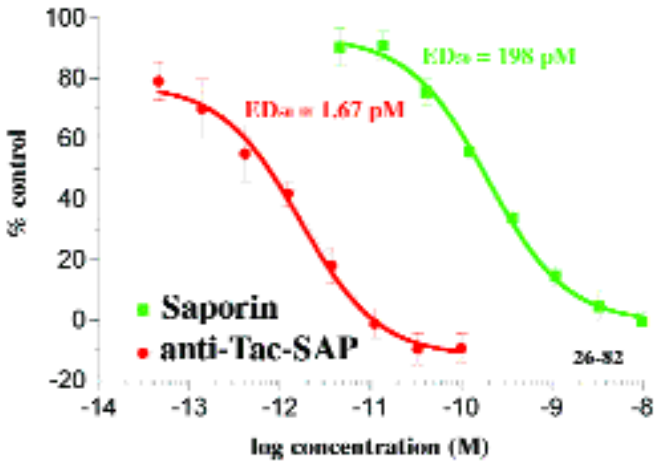


Figure 2. Cytotoxicity of anti-Tac-SAPand of non-conjugated saporin (Saporin) to SR cells in culture. Cells were plated at 5000 cells per well and allowed to acclimate. Samples were added at the indicated concentrations and cells were incubated for 72 hours. MTS (Promega) was added and, after color development, wells were read with a Molecular Dynamics SpectraMax 340. ED_{50}'s of each compound are color-coded. Data analysis is by PRISM (GraphPad).

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Targeting Technology

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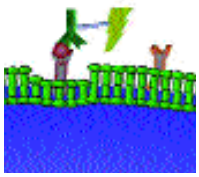
[§]or growth factor, peptide, ligand, or cytokine

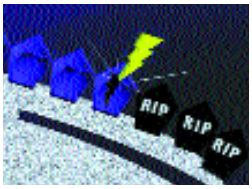
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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 this issue's cover article.

LEAMMOY ☐☐☐☐☐☐☐

CYTOPEN ☐☐☐☐☐☐☐

JOTBIVECE ☐☐☐☐☐☐☐☐☐

PYRHETA ☐☐☐☐☐☐☐

DRENBU ☐☐☐☐☐☐

HOW THE FEMALE BIOLOGY STUDENT RESPONDED WHEN ASKED TO BE HIS LAB PARTNER

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

Answer:

SHE ☐☐☐☐☐☐

AT THE ☐☐☐☐☐☐

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Limit one entry per laboratory.

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2. Fax in this entire page with the correct solution by August 31, 2002.
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See last quarter's winners, page 6.

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Oct-Nov-Dec 2002
Volume 3, Issue 4

Targeting Trends

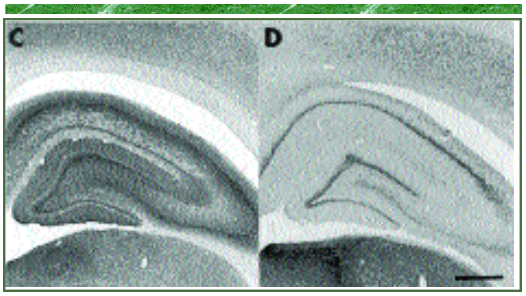
Reporting the latest news in Molecular Surgery

Does 192-IgG-Saporin or 5,7-DHT kill cognitive functions in the rat?

Contributed by Jean-Christophe Cassel, PhD, Laboratoire de Neurosciences Cognitives et Comportementales, UMR 7521 Université Louis Pasteur/CNRS, Strasbourg, France & Institut für Experimentelle und Klinische Pharmakologie und Toxikologie der Universität Freiburg, Neuropharmakologisches Labor, Freiburg, Germany. Dr. Cassel and colleagues are the authors of four publications released this quarter using 192-Saporin.

Trying to understand how various neuroanatomically- and/or neurochemically-defined systems of the brain contribute to elaborate functional outputs at an integrated level – including behavior – roughly relies on two types of approaches. The first consists of exploring brain regions while functional outputs are prepared or expressed (*e.g.* EEG). The second approach uses more invasive tools (*e.g.* lesions), alters brain structures, pathways or transmitter systems, and measures functional consequences. One problem with the second approach concerns the neuroanatomical and/or neurochemical selectivity. The more selective the lesion of a particular system in the brain, the more a functional alteration can be linked to the operations enabled by the system. 192 IgG-Saporin has emerged as the most selective toxin to lesion the cholinergic neurons of the basal forebrain. As such, this immunotoxin enables exploration of the contribution to cognitive functions of the septohippocampal and basalocortical cholinergic projection systems while considering the "cholinergic hypothesis of geriatric dysfunctions" proposed by Bartus in 1982.[1] From the first behavioral studies with 192 IgG-Saporin lesions, it became evident that cognitive deficits were not as large as expected from previous studies relying on less selective

(continued on page 6)



AChE-positive staining patterns in occipital and parietal cortices surrounding hippocampus. Note decrease in staining in rats treated with 192-IgG-Saporin and 5,7-DHT(D) as opposed to sham (C).
Scale bar, 750 µm
Adapted from Lehmann *et al.* (2002) *Eur J Neurosci* 15:1991-2006.
Printed with permission from the Federation of European Neuroscience Societies

SP-SAP Efficacy Reported at IASP 10th World Congress on Pain

Clinicians and scientists alike were talking about SP-SAP at the August meeting of the International Association for the Study of Pain. During the pre-meeting Refresher Courses, Drs. Alan Basbaum, Patrick Mantyh, and Stephen Hunt explained to an audience of mainly physicians how SP-SAP eliminates neurons that send the chronic pain signal to the brain. Scientists from five different laboratories presented their findings on SP-SAP and other ATS products during poster and slide presentations (see page 5).

Cell Targeting Technologies is currently seeking investors to take SP-SAP into clinical trials for the treatment of chronic pain.

Inside this issue:

Newsletter Highlights

- Custom Conjugations *(page 2)*
- Abstracts from FENS *(page 5)*
- Abstracts from IASP *(page 5)*
- anti-SERT-SAP *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Unilateral lesions of the cholinergic basal forebrain and fornix in one hemisphere and inferior temporal cortex in the opposite hemisphere produce severe learning impairments in rhesus monkeys.

Easton A, Ridley RM, Baker HF, Gaffan D
Cereb Cortex 12:729-736, 2002

The authors used a combination of basal forebrain lesioning using ME20.4-SAP (Cat. #IT-15) and surgery to isolate the inferior temporal cortex and medial temporal cortex from cholinergic afferents in rhesus monkeys. Testing of the treated animals demonstrated severe impairments in learning visual scenes and object-reward associations.

Selective lesion of cholinergic neurons in the medial septum by 192 IgG-saporin impairs learning in a delayed matching to position T-maze paradigm.

Johnson DA, Zambon NJ, Gibbs RB
Brain Res 943(1):132-141, 2002

The authors investigated the effects of selective cholinergic depletion in the medial septum on a spatial memory (DMP) task. Direct infusion of 0.22 or 1.0 μg 192-Saporin (Cat. #IT-01) produced a near complete depletion of cholinesterase-positive neurons for either dose. The DMP task provides a sensitive behavioral assay for deficits in cholinergic projections.

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

Hippocampal brain-derived neurotrophic factor gene regulation by exercise and the medial septum.

Berchtold NC, Kesslak JP, Cotman CW
J Neurosci Res 68(5):511-521, 2002

Brain-derived neurotrophic factor (BDNF) enhances neuron function and plasticity. The authors lesioned rats with medial septal injections of 192-Saporin (Cat #IT-01, 375 ng in 0.5 μl PBS) or OX7-SAP (Cat #IT-02, 12.5 or 25 ng in 0.5 μl PBS). 192-Saporin affected the sedentary, but not exercise-induced levels of BDNF. OX7-SAP reduced levels in both groups in a dose-dependent manner.

Grafts of fetal septal cells after cholinergic immunotoxic denervation of the hippocampus: a functional dissociation between dorsal and ventral implantation sites.

Cassel JC, Gaurivaud M, Lazarus C, Bertrand F, Galani R, Jeltsch H
Neuroscience 113(4):871-882, 2002

The authors lesioned rats with intraseptal infusions of 0.8 μg 192-Saporin (Cat. #IT-01), then implanted fetal cells in either the dorsal or ventral hippocampus. Only grafts into the dorsal hippocampus counteracted the effect of cholinergic lesions on spatial working memory performance.

Inhibition of neuropathic pain by selective ablation of brainstem medullary cells expressing the μ-opioid receptor.

Porreca F, Burgess SE, Gardell LR, Vanderah TW, Malan TP Jr, Ossipov MH, Lappi DA, Lai J
J Neurosci 21(14):5281-5288, 2001

The presence of descending projections in the pain pathway raises the possibility that abnormal sustained activity may perpetuate chronic pain. Using 3-ng injections of dermorphin-SAP (Cat #IT-12) on either side of the RVM in rats the authors both prevented and reversed neuropathic pain caused by spinal nerve ligation.

Identification of a potential ejaculation generator in the spinal cord.

Truitt WA, Coolen LM
Science 297(5586):1566-1569, 2002
(Also see pp 1460-1461 for News of the Week)

The authors lesioned a specific population of rat spinothalamic neurons using 6-8 injections of 4 ng SSP-SAP (Cat. #IT-11). Whereas the treated rats exhibited no change in sexual behavior such as mounts and intromissions, ejaculatory behavior was completely abolished. The data suggest that this population of neurons may function as an ejaculation generator in the spinal cord.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Immunotoxic catecholamine lesions attenuate 2DG-induced increase of AGRP mRNA.

Fraley GS, Dinh TT, Ritter S
Peptides 23(6):1093-1099, 2002

The authors investigated mRNA levels of both agouti gene-related protein (AGRP) and neuropeptide Y (NPY) in rats after lesioning the PVH with anti-DBH-SAP (42 ng in 200 nl, Cat. #IT-03). The results show that the increase in AGRP mRNA levels due to 2DG administration was completely blocked.

Cholinergic depletion by IgG 192-saporin retards development of rat barrel cortex.

Zhu XO, de Permentier PJ, Waite PM
Brain Res Dev Brain Res 136:1-16, 2002

It has been shown that cholinergic afferents from the basal forebrain are necessary for normal cortical morphogenesis. However, the role of these projections in the development of the thalamocortical topographical map has not been investigated. Using the facial whisker barrel field in the rat somatosensory cortex as a development model, the authors administered 192-Saporin to newborn pups (0.1 μg, Cat. #IT-01). The data show a transient delay in the development of the barrel pattern over the first postnatal week.

Reduced aversion to oral capsaicin following neurotoxic destruction of superficial medullary neurons expressing NK-1 receptors.

Simons CT, Gogineni AG, Carstens MI, Carstens E
Brain Res 945:139-143, 2002

Capsaicin-induced irritation of the dorsal anterior tongue is mediated by nociceptors expressing VR-1 receptors. The role of NK-1 receptor-expressing neurons during the ingestion of capsaicin was examined by injecting 20 μl of 2.27 μM SP-SAP (Cat. #IT-07) into the cisterna magna of rats. Lesioned rats consumed significantly more water containing high concentrations of capsaicin than control animals.

Spinal Noradrenergic Activation Mediates Allodynia Reduction from an Allosteric Adenosine Modulator in a Rat Model of Neuropathic Pain

Li X, Conklin D, Ma W, Zhu X, Eisenach JC
Pain 97:117-125, 2002

T62 is a thiobene compound that enhances adenosine agonist binding to the A1 receptor. Activation of the adenosine receptor has been effective in several different pain models. The authors used a spinal nerve ligation model for mechanical allodynia to assess T62 efficacy and mode of action. Rats treated with anti-DBH-SAP (4 μg in 5 μl, Cat. #IT-03) experienced no anti-allodynia effects from T62 administration, indicating that modulation of mechanical allodynia by T62 utilizes the spinal noradrenergic system.

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

Congratulations to J.C. Cassel and collaborators for having five scientific publications this past quarter! Listed below are brief summaries of four of these articles. See page 3 for a review of one of them and this issue's cover story for more information about their interesting research.

Effects of 192 IgG-saporin on acetylcholinesterase histochemistry in male and female rats.

Galani R, Jeltsch H, Lehmann O, Bertrand F, Cassel JC
Brain Res Bull 58(2):179-186, 2002
Male rats were treated with estradiol, and 2-μg i.c.v. injections of 192-Saporin (Cat #IT-01).

Selective immunolesions of CH4 cholinergic neurons do not disrupt spatial memory in rats.

Galani R, Lehmann O, Bolmont T, Aloy E, Bertrand F, Lazarus C, Jeltsch H, Cassel JC
Physiol Behav 76:75-90, 2002
Rat NBM was lesioned using 0.2 or 0.4 μg of 192-Saporin (Cat. #IT-01).

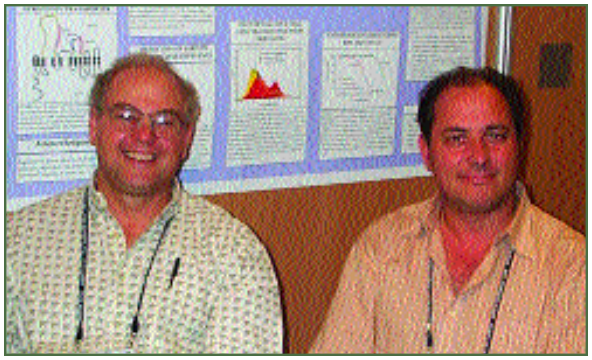
Combined 192 IgG-Saporin and 5,7-dihydroxytryptamin lesions in the male rat brain: a neurochemical and behavioral study.

Lehmann O, Jeltsch H, Lazarus C, Tritschler L, Bertrand F, Cassel JC
Pharm Biochem Behav 72:899-912, 2002
Injections of 1 μg per ventricle of 192-Saporin (Cat. #IT-01).

5,7-DHT-induced hippocampal 5-HT depletion attenuates behavioural deficits produced by 192 IgG-saporin lesions of septal cholinergic neurons in the rat.

Lehmann O, Bertrand F, Jeltsch H, Morer M, Lazarus C, Will B, Cassel JC
Eur J Neurosci 15(12):1991-2006, 2002
The authors used 192-Saporin, 0.4 μg/side intraseptal (Cat. #IT-01).

Forum of European Neuroscience (FENS): July 13-17, Paris France



Doug Lappi and Giampiero Leanza discuss 192-Saporin research in the ATS booth at the FENS meeting in Paris.

Differentialcontribution ofthe cholinergic basalnuclei to attentionand spatialmemory in the rat.
Lehmann O [1], Grottick AJ [2], Cassel JC [1], Higgins GA[2]
[1] LN2C, UMR 7521 CNRS-ULP, Strasbourg, France; [2] PRBN Hoffmann La Roche, Basel, Switzerland
Featuring 192-Saporin (Cat. #IT-01)

Effects of mice EGF-responsive neuralstem cells graftsand fetal septalcells grafts implanted into the dorsalhippocampus ofrats after immunotoxic denervation.
Jeltsch H [1], Aloy E [1], Schimchowitsch S [1], Caillard S [2], Mohier E [3], Cassel JC [1]
[1] LN2C-UMR7521, Strasbourg, France; [2] Serv Néphrol, CHU, Strasbourg, France; [3] UPR 1352, Strasbourg, France
Featuring 192-Saporin (Cat. #IT-01)

Short and long term-effects on the serotonergic system of a selectivecholinergic lesion in rats.
Ramirez MJ, Garcia-Alloza M, Lasheras B
Dept Pharmacol, Univ Navarra, Pamplona, Spain
Featuring 192-Saporin (Cat. #IT-01)

Selective neonatallesions ofthe basalforebrain cholinergic neurons impair memory ofsocially-transmitted food preferences in adult rats.
Ricceri L[1], Moles A [2], Scattoni ML[1], Calamandrei G [1]
[1] Sect Comp Psychol Lab FOS ISS Rome, Italy; [2] Psychobiol Psychopharmacol Inst CNR Rome, Italy
Featuring 192-Saporin (Cat. #IT-01)

Selective lesioning ofthe developing cholinergicand noradrenergic systems: Anatomical, neurochemicaland functional effects.
Leanza G [1], Cataudella T [2]
[1] Dept. Physiol and Pathol, Trieste, Italy; [2] Dept Physiol Sci, Catania, Italy
Featuring Anti-DBH-SAP(Cat. #IT-03)

Impairmentand restoration ofspatialabilities following 192 IgG-saporin or quisqualicacid lesions ofthe median septum in rats.
Brandner C, *Inst Physiol Lausanne, Switzerland*
Featuring 192-Saporin (Cat. #IT-01)

Targeting serotonin re-uptake transporter (SERT) -expressing cells with a monoclonalantibody to an epitope from the extracellular domain of SERT: Results with a saporin conjugate.
Lappi D [1], Kohls M [1], Majer K [1], Russell B [1], Blakely R [2], Richerson G [3]
[1] Advanced Targeting Systems; [2] Vanderbilt University; [3] Yale University
Featuring Anti-SERT (Cat. #AB-N09) and anti-SERT-SAP (Available Soon, see page 7 "Featured Products")

IASP 10th World Congress on Pain: August 17-22, San Diego California

Descending facilitation from the rostral ventromedialmedullamaintains, but does not initiate, neuropathic pain.
Burgess SE, Gardell LR, Ossipov MH, Malan T, Vanderah TW, Lai J, Porreca F
Pharmacology and Anesthesiology, University of Arizona, Tucson, AZ
Featuring Dermorphin-SAP(Cat. #IT-12)

Loss of IB4 staining in dorsalroot ganglion neuronsafter spinalnerve ligation is not the result ofcelldeath.
Arunkumar R, Ackerman LL, Jones III R, Holdsworth R, Proudfit HK, Hammond DL
Anesthesia, Neurosurgery and Pharmacology, Univ of Iowa, Iowa City, IA
Featuring IB4-SAP(Cat. #IT-10)

Loss of IB4-positive sensory neurons mitigates the consequences ofnerve injury in the rat.
Tarpley JW, MacIntyre E, Martin WJ
Pharmacology, Merck Research Labs, Rahway, NJ
Featuring IB4-SAP(Cat. #IT-10)

Role oflamina I neurons expressing the substance p receptor in the prevention and treatment of a spontaneous pain-like behavior following excitotoxic spinal cord injury (SCI).
Yezierski RP[1], Yu C [1], Lappi DA[3], Mantyh PW [4], Wiley RG [2]
[1] Orthodont & Neurosci, Univ Florida, Gainesville, FL; [2] Neurol Serv, VAMC, Nashville, TN; [3] Advanced Targeting Systems, San Diego, CA; [4] Neurosystems Center, University of Minnesota, Minneapolis, MN
Featuring SSP-SAP(Cat. #IT-11)

Plasticityofwide dynamic range neurones following site-selectiveablation of NK-1 receptor expressing lamina I neurones in rat spinalcord.
Suzuki R [1], Morcuende S [2], Webber M [2], Hunt SP [2], Dickenson AH [1]
[1] Pharmacology, Univ College London, London, United Kingdom; [2] Anatomy and Developmental Biology, Univ College London, London, United Kingdom
Featuring SP-SAP(Cat. #IT-07)

Does 192 IgG-Saporin or 5,7-DHT kill cognitive functions?

(continued from page 1)

lesions: whether administered intracerebroventricularly (i.c.v.) or intraparenchymally, extremely large cholinergic lesions seemed required to induce clear-cut, though submaximal deficits. There are certainly several ways to look on such data,[2] and at least one question worth mentioning: wouldn't other neurotransmitter systems also participate in those behavioral capabilities commonly attributed to cholinergic functions?[3] This question led us to carry out the first experiments in which 192 IgG-Saporin lesions of basal forebrain cholinergic neurons were combined to 5,7-DHT lesions of the ascending serotonergic projection system. In this study, the toxins were injected, separately or in combination, in the ventricles of female rats.[4] While none of the single lesions induced any clear-cut behavioral deficit, the combination of both toxins induced a marked alteration of spatial working memory in a radial maze. Part of these results were recently confirmed in male rats.[5] In another study,[6] the immunotoxin was injected into the septal region and 5,7-DHT into the fimbria-fornix/cingular bundle pathways to achieve neurochemical and neuroanatomical selectivity at once. The cholinergic lesion was large enough to induce a severe deficit in a radial-maze working memory task. Interestingly, damage to the serotonergic innervation of the hippocampus, which had no proper effect, reduced the saporin-induced deficit, suggesting that cholinergic and serotonergic mechanisms interactively contribute to the control of spatial working memory capabilities. These findings also consolidated the idea that reducing the serotonergic tone in the hippocampus may attenuate the deficits produced by a cholinergic denervation of this structure.

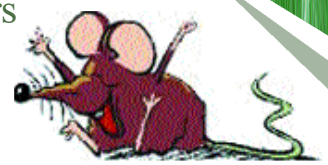
What about the nucleus basalis magnocellularis (NBM), another major cholinergic nucleus in the brain with cognitive implications? Again using 192 IgG-Saporin, we induced gradual lesions of NBM cholinergic neurons and ran our rats through a series of spatial tasks in which rats subjected to non-selective NBM lesions were previously shown to exhibit spatial memory impairments.[7] Surprisingly, despite extensive denervation of cortical areas and utilization of a radial-maze testing protocol adding a strong temporal load to the task (trial interruption by delay of up to 6 hours), we were unable to show any working- or reference-memory deficit.[7] These findings demonstrated that the basalocortical cholinergic system did not play a crucial role in such mnemonic operations. This conclusion was consolidated in another study by Lehmann, Grottick, Cassel and Higgins (in preparation), in which 192 IgG-Saporin was injected into the septal region and/or the NBM. Rats were tested in a 5-choice serial reaction test (5-CSRT) assessing visual attention and, subsequently, in a radial maze. Rats with septal lesions performed normally in the 5-CSRT, but were dramatically impaired in the radial maze. Rats with NBM lesions were markedly impaired in the 5-CSRT, but performed normally in the radial maze. Rats with both lesions were impaired in both tasks.

Our results show that 192 IgG-Saporin causing cholinergic denervation of the hippocampus (intraseptal) alters spatial memory processes, and denervation of the cortex (NBM) alters attention. At least in the hippocampus, there is evidence that attenuated serotonergic tone contributes to reduce the magnitude of spatial working memory deficits due to the cholinergic damage. Although this is probably not the only mechanism involved, we have recently found that a 5,7-DHT-induced serotonergic denervation of the hippocampus could facilitate the electrically-evoked release of acetylcholine from hippocampal slices, and that this facilitation was abolished by intrahippocampal grafts rich in serotonergic neurons.[8] Thus, 192 IgG-Saporin kills cholinergic neurons and, under circumstances, cognitive functions. Depending on how the lesion is produced, it seems that killing serotonergic neurons may be an inimical collaborator or a benevolent disparager as to the cognitive malevolence of 192 IgG-Saporin.

REFERENCES

1. Bartus RT, Dean RL3rd, Beer B, Lippa AS (1982) *Science* 217: 408-414.
2. Cassel JC, Jeltsch H (1995) *Neurosci* 69:1-41.
3. Wrenn CC, Wiley RG (1998) *Int J Dev Neurosci* 16:595-602.
4. Lehmann O, Jeltsch H, Lehnardt O, Pain L, Lazarus C, Cassel JC (2000) *Eur J Neurosci* 12:67-79.
5. Lehmann O, Jeltsch H, Lazarus C, Tritschler L, Bertrand F, Cassel JC (2002) *Pharmacol Biochem Behav* 72:899-912, 2002.
6. Lehmann O, Bertrand F, Jeltsch H, Morer M, Lazarus C, Will B, Cassel JC (2002) *Eur J Neurosci* 15:1991-2006.
7. Galani R, Lehmann O, Bolmont T, Aloy E, Bertrand F, Lazarus C, Jeltsch H, Cassel JC (2002) *Physiol Behav* 76:75-90.
8. Birthelmer A, Schweizer T, Jeltsch H, Jackisch R, Cassel JC (In Press) *Eur J Neurosci*.

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: MYELOMA POTENCY OBJECTIVE THERAPY BURDEN

Answer: How the female biology student responded when asked to be his lab partner — SHE JUMPED AT THE CHANCE

WINNERS: Bruce Pappas, Carleton University, Life Science Res Ctr * Lynn Young, RW Johnson PRI * Javier Mestas, UC Irvine, Bio Sci II * Kenira Thompson, Univ of Texas, San Antonio * Marta de Jesus, Ventura College * Sela James, Catholic University of America

Targeting Tools: Featured Products

anti-SERT-SAP

At the Society for Neuroscience (SFN) meeting, November 2-7 in Orlando FL, Advanced Targeting Systems will announce the availability for purchase of its newest neuron-specific cytotoxin, anti-SERT-SAP. This immunotoxin utilizes a monoclonal antibody to the third extracellular domain of the serotonin re-uptake transporter (SERT) that was described at last year's SFN meeting, as were *in vitro* data on the immunotoxin activity.

At the Federation of European Neuroscience Societies meeting in Paris in April, Lappi and colleagues presented a poster demonstrating the specificity of this antibody for cells that express SERT, and the lack of reactivity for cells that express the norepinephrine transporter or the dopamine transporter.

Figures 1a and 1b (provided by E. Nattie) show the *in vivo* activity of anti-SERT-SAP against SERT-bearing neurons of the raphe nucleus; there is an almost complete removal of the target cell population, while the architecture of the raphe is maintained beautifully. The potential of anti-SERT-SAP for the understanding of the functional roles of serotonergic neurons in behavior is enormous. This immunotoxin continues the tradition of Advanced Targeting Systems in bringing you the highest quality materials with the most rigorous quality control; it has been in development for more than two years and has been carefully characterized, including sending it out for evaluation to leading scientists, as we do with other ATS products.

5,7-DHT has often been used for the lesioning of serotonergic neurons (see Dr. Cassel's excellent cover article in this issue). We would like to emphasize the differences and advantages of the new anti-SERT-SAP. Because 5,7-DHT also targets noradrenergic neurons, pre-injection of a protective agent such as desipramine is required. In addition, 5,7-DHT is rapidly oxidized, requiring even a third compound (usually ascorbic acid) to be injected. Finally, there is a significant difference in the mode of action. 5,7-DHT will lesion axons and terminals and usually leave a cell that can recover (Baumgartner & Bjorklund, *Annu Rev Pharmacol Toxicol,* 1976, 16:101-111), whereas anti-SERT-SAP will permanently eliminate the target cell and any function it may perform.

At the 2002 SFN meeting, Dr. Eugene Nattie will make a slide presentation showing the *in vivo* efficacy of this targeted toxin and its specificity for serotonergic neurons (Program #221.3, Monday, 8:30 AM, Nov. 4, Room 206a). If you can't make that, stop by and visit the ATS booth (#1616). We'll give you all the details.

Coming Soon!

anti-DAT-SAP
Targeted Toxin

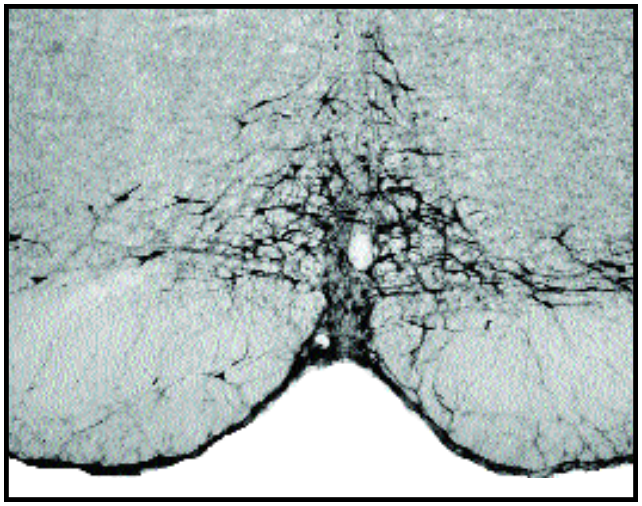


Figure 1a. The control rat brain section, stained for TPOH, received no injection of anti-SERT-SAP.

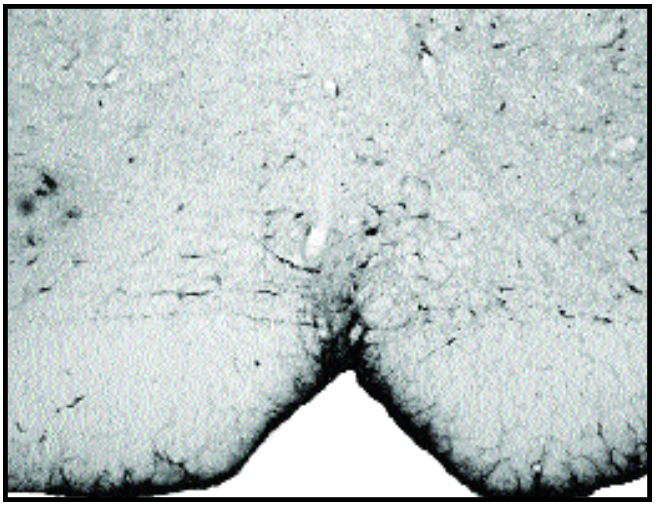


Figure 1b. The anti-SERT-SAP treated rat received two adjacent 100-nl injections of anti-SERT-SAP (1 μM) into medullary raphe at the level of the caudal part of facial nucleus. The injections were 2 weeks prior to sacrifice. Treated rats had up to a 16% reduction in the ventilatory response to breathing 7% CO_2 during both sleep and wakefulness. These data show that TPOH ir neurons in the medullary raphe are involved in the ventilatory response to systemic hypercapnia.

Visit the ATS website for a complete list of products.

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.

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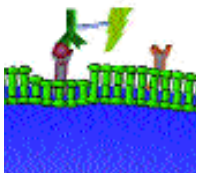
[§]or growth factor, peptide, ligand, or cytokine

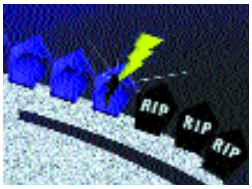
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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.

LAULLERC

RADLOS

LEANNOTA

RHIDBY

ROWLE

WHAT THE CLONERS SAID WHEN THEY ENTERED THE BARNYARD.

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

Answer:

_____ _____.
_____!

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, 2002.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

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