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Targeting Trends

Reporting the latest news in Molecular Surgery

Deletion of NPY/AGRP and POMC Neurons in the Arcuate Nucleus by Leptin-Saporin Produces Hyperphagia, Obesity and Changes in Diurnal Feeding Patterns in Rats

Contributed by Ai-Jun Li, Qing Wang, Thu T. Dinh and Sue Ritter
Programs in Neuroscience, Washington State University, Pullman, Washington 99164-6520, USA.

Leptin is a fat tissue-derived hormone with widespread actions in brain and peripheral tissues. Leptin's actions are mediated by the long isoform of the leptin receptor, OB-Rb, which has a lengthy intracellular region containing several motifs required for signal transduction via the JAK2/STAT3 pathway. Leptin profoundly influences food intake and body weight, in large part by its actions on OB-Rb receptors in the arcuate nucleus (Arc) in the basomedial hypothalamus. Two populations of neurons in the Arc that are importantly involved in these functions are neuropeptide Y (NPY) and agouti gene-related protein (AGRP) co-expressing neurons and pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) co-expressing neurons. The majority of both NPY/AGRP and POMC/CART neurons are OB-Rb-positive. Exogenous administration of leptin excites and increases phospho-STAT3 expression in POMC/CART neurons and inhibits NPY neurons in the Arc.

In the present study, we used a novel leptin-saporin conjugate (Lep-SAP), a targeted toxin developed recently by Advanced Targeting Systems, to lesion OB-Rb-expressing NPY/AGRP and POMC/CART neurons in Arc.

(continued on page 6)

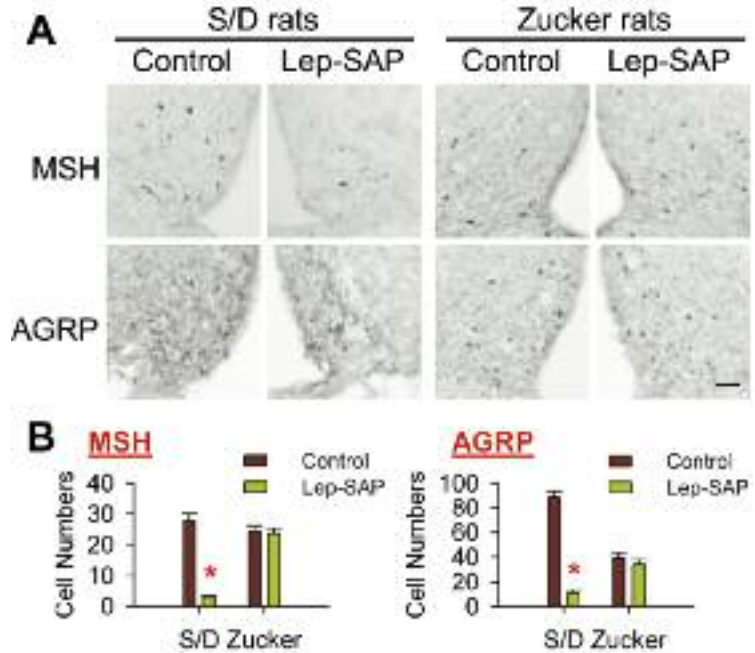


Figure 1.
Effects of unilateral Lep-SAP injection into Arc on α-MSH and AGRP expression.
(A) Representative photomicrographs of α-MSH and AGRP cell bodies after unilateral injection of Lep-SAP into Arc in Sprague Dawley (S/D) or Zucker *fa/fa* (Zucker) rats.
(B) Numbers of α-MSH- and AGRP-positive cell bodies in the Arc of S/D and Zucker rats ipsilateral and contralateral to Lep-SAP injection. In S/D rats, but not Zucker *fa/fa* rats, Lep-SAP significantly decreased the numbers of α-MSH- and AGRP-positive cells in Arc on the injected side, compared to the uninjected side.
* $P < 0.01$; unpaired t-test vs. control non-injection side.
Calibration bar = 100 µm.

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

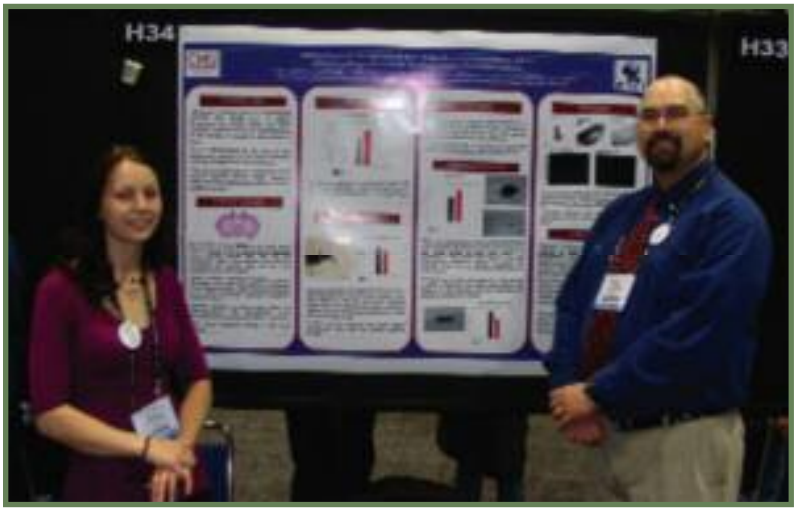
2009 Society for Neuroscience Poster Award Winner

By Douglas A. Lappi

We are pleased to announce the winner of the Poster of the Year at the Society for Neuroscience meeting: Ai-Jun Li of Washington State University, who presented the work on behalf of himself and his colleagues, Q. Wang, T.T. Dinh and Sue Ritter. The poster was entitled, *"Leptin-saporin injection into the arcuate nucleus lesions NPY/AGRP and POMC neurons and produces hyperphagia, obesity and changes in diurnal feeding patterns in rats."* The work presented the activity of Leptin-SAP (Cat. #IT-47) in feeding in a clever manner, utilizing the Zucker *fa/fa* rat, which has no functional leptin receptor, as well as Blank-SAP, which has no target receptor, but a similar structure. We congratulate Ai-Jun Li and his collaborators in prevailing over several outstanding posters. The cover article in this issue is written by Dr. Li and explains their very interesting work with Leptin-SAP.

In addition to having his work featured on the cover, Dr. Li also receives a $500 product credit, an autographed copy of "Molecular Neurosurgery With Targeted Toxins," and a variety of ATS promotional products.

Dr. Ai-Jun Li and Dr. Douglas Lappi in the ATS booth at the Society for Neuroscience meeting in Chicago.



J.J. Matchynski and colleague at their poster presentation using mu p75-SAP.

R. P. Gaykema, G. C. Thacker, N. J. Shapiro, L. E. Goehle, Ctr. for the Study of Complementary and Alternative Therapies, Univ. Virginia Sch. of Nursing, Charlottesville, VA.

This striking poster demonstrated an amazing loss of symptoms, "sickness behavior," that were suspected of being due to noradrenergic/adrenergic input by the use of anti-DBH-SAP (CAT. #IT-03). The demonstration of sickness behavior having a neuronal underpinning was a fascinating surprise to us, but not to them.

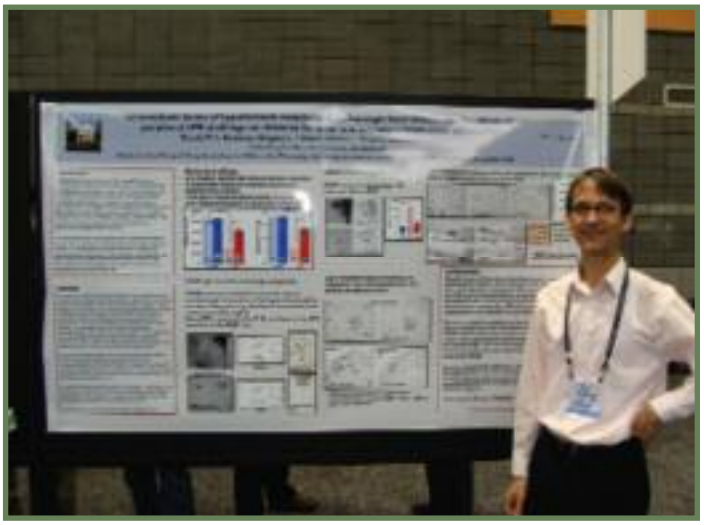
There were many posters at this year's meeting and we are always very interested to see how our clever and talented customers have used the ATS targeting technology. We look forward to next year's meeting in San Diego.

Visit our website to see a complete listing of abstracts submitted for presentation at the 2009 Society for Neuroscience meeting. (http://www.atsbio.com/news/09_sfnabs.html)

One of the other contenders for the Award was: "*Intracerebroventricular injections of mu-P-75 saporin can produce memory deficits without impairing motor deficits in a mouse model of Alzheimer's disease."* J. J. Matchynski, S. Lowrance, J. Rossignol, N. Puckett, N. Derkorver, J. Radwan, K. Trainor, M. Sandstrom, G. Dunbar, Central Michigan Univ., Mount Pleasant, MI.

The group from Central Michigan presented nice work on memory deficits in mice after loss of basal forebrain cholinergic neurons due to treatment with mu p75-SAP (Cat. #IT-16). This work suggests the ability to do analysis of cholinergic-deprived animals that can have all sorts of genetic knock-ins and knock-outs.

The third contender was: "*Immunotoxic lesion of hypothalamic noradrenergic/adrenergic input ameliorates the effects of peripheral LPS challenge on sickness behavior and associated brain c-Fos expression."*



R.P. Gaykema presents his surprising results using anti-DBH-SAP.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Neuroprotective effects of testosterone on dendritic morphology following partial motoneuron depletion: efficacy in female rats

Wilson RE, Coons KD, Sengelaub DR
Neurosci Lett 465(2):123-127, 2009.

Previous work demonstrated a protective effect from testosterone in a motoneuron nerve injury model for male rats. This work investigated whether testosterone has the same effect in females. Female rats received 2 µg of CTB-SAP (Cat. #IT-14) into the left vastus medialis muscle. 4 weeks later surviving motoneurons were visualized with CTB conjugated to HRP. Testosterone treatment greatly attenuated the atrophy seen in control animals, suggesting that testosterone is also a neurotherapeutic agent in females.

CTB-SAP
a chemical conjugate of the cholera toxin B-subunit and saporin

Proteomic analysis uncovers novel actions of the neurosecretory protein VGF in nociceptive processing

Riedl MS, Braun PD, Kitto KF, Roiko SA, Anderson LB, Honda CN, Fairbanks CA, Vulchanova L
J Neurosci 29(42):13377-13388, 2009.

Peripheral tissue injury can alter protein expression in sensory neurons, which may contribute to abnormal nociceptive processing. The authors used cultured dorsal root ganglion neurons as a model for axotomized neurons to examine early changes in protein expression after nerve injury. Several different parameters were measured, including immunohistochemistry using anti-TrkA (Cat. #AB-N03). The data show an increased level of a putative neuropeptide precursor, VGF, as a result of nerve injury.

This antibody recognizes rat trkA (high affinity nerve growth factor receptor). Anti-trkA was developed in rabbit using the extracellular fragment from rat trkA (amino acids 1-416); purified by protein A chromatography.

Amyloid-beta expression in retrosplenial cortex of triple transgenic mice: relationship to cholinergic axonal afferents from medial septum

Robertson RT, Baratta J, Yu J, LaFerla FM
Neuroscience 164(3):1334-1346, 2009.

In this work the authors developed a model to examine the relationship between afferent projections and the formation of amyloid-beta (Aβ) deposits. Mice received 1.86-µg unilateral injections of mu p75-SAP (Cat. #IT-16) into the lateral ventricle. Lesioned animals had persistent Aβ immunoreactivity in layer III of the granular division of retrosplenial cortex (RSg). This data indicates that septal cholinergic axonal projections transport Aβ or amyloid precursor protein to layer III of the RSg.

mu p75-SAP
a chemical conjugate of the affinity-purified rabbit polyclonal antibody p75NTR (Cat. #AB-N01AP) and saporin

Serotonin Transport and Metabolism in the Mammary Gland Modulates Secretory Activation and Involution

Marshall AM, Nommsen-Rivers LA, Hernandez LL, Dewey KG, Chantry CJ, Gregerson KA, Horseman ND
J Clin Endocrinol Metab 2009.

Serotonin is known to be a local regulator of lactation homeostasis. This work examined the roles of the serotonin reuptake transporter (SERT) and monoamine oxidase in this system. Immunohistochemical and immunocytochemical staining was done on human primary mammary epithelial cells and mouse tissue with a SERT antibody (Cat. #AB-N09). Additional data included epidemiological studies and selective serotonin reuptake inhibitor treatment of mice. The results suggest that women taking SSRI inhibitor medications were more likely to experience delayed secretory activation.

This antibody recognizes cells that express SERT in rat, human, and mouse. The immunogen is a peptide from the fourth extracellular domain of the rat SERT. This antibody was produced in tissue culture supernatants. The antibody is routinely tested by flow cytometry.

Nitrous oxide-induced analgesia does not influence nitrous oxide's immobilizing requirements

Jinks SL, Carstens E, Antognini JF
Anesth Analg 109(4):1111-1116, 2009.

Noradrenergic neurons in the locus coeruleus (LC) are involved with the analgesic action of nitrous oxide (N_2O). In order to examine whether these neurons are also involved with the immobilizing effects of N_2O, rats received 4-µg intracerebroventricular injections of anti-DBH-SAP (Cat. #IT-03). Mouse IgG-SAP (Cat. #IT-18) was used as a control. Lesioned animals did not experience the analgesic effects of N_2O, but the

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(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

immobilizing effects were still present. The data demonstrate that the immobilizing mechanism of N_2O is independent from its analgesic effects.

Anti-DBH-SAP
a chemical conjugate of the mouse monoclonal antibody to dopamine beta-hydroxylase and saporin
target: cells that express dopamine beta-hydroxylase

Spatial memory following selective cholinergic lesion of the nucleus basalis magnocellularis

Dashniani M, Burjanadze M, Beselia G, Maglakelidze G, Naneishvili T
Georgian Med News 174):77-81, 2009.

This study investigated the role of cholinergic nucleus basalis magnocellularis (NBM) cells in learning and memory. Rats received bilateral 200-ng injections of 192 IgG-SAP (Cat. #IT-01) into the NBM. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The results indicate the NBM is important in accurate spatial learning and processing information about the spatial environment. Deficits in rats with the cholinergic lesion may be due to lowered attentional function.

192-IgG-SAP
a chemical conjugate of a mouse monoclonal antibody to rat $p75^{NTR}$ and saporin
target: LNGFR-positive cells in rat

Mouse IgG-SAP
a chemical conjugate of pre-immune mouse IgG antibody and saporin

Cell transplantation: a future therapy for narcolepsy?

Arias-Carrion O, Murillo-Rodriguez E
CNS Neurol Disord Drug Targets 8(4):309-314, 2009.

This review covers the current understanding of narcolepsy and discusses the potential for transplants as a therapeutic treatment. Animal models are summarized, including the use of orexin-SAP (Cat. #IT-20) in rats. The review goes on to suggest that production of orexigenic neuroblasts from stem cells may be a useful therapy.

Orexin-SAP has been discontinued.

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: ITCH
HISTMAMINE
SCRATCH
MUTANT
INTENSE

Answer: He... "MIST" THEM.

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

WINNERS: Kim Van Vliet, Univ Florida, Gainesville, FL * Michael Bienkowski, Univ Pittsburgh, Pittsburgh, PA * April Price, Univ California, San Francisco, CA * Michael Fenko, NYS Institute for Basic Research, Staten Island, NY * Jean Peduzzi, Wayne State Univ SOM, Detroit, MI * Richard Fuerstenberg, R&D Systems, Inc, Minneapolis, MN * Julienne Mullaney, Univ Maryland SOM, Baltimore, MD * Glenn Kageyama, Cal Poly Pomona Univ, Pomona, CA * Richard Robertson, UC SOM, Irvine, CA * Seto Chice, SUNY-HSC, Brooklyn, NY * Heidi Day, Univ Colorado, Boulder, CO * Amalia Dingman, Norman, OK * Darlene Martineau, Del Mar, CA * Ruth Stornetta, Univ Virginia, Charlottesville, VA * Dr. Roger Guillemin, Salk Institute, San Diego, CA

Solve the Teaser online at: www.atsbio.com/news/10q1_teaser.html

The Importance of Product References

A message from the Editor

As we start the new year, I'd like to thank all our customers for the top quality science that has resulted in so many important and innovative research publications. Advanced Targeting Systems is dedicated to providing targeting reagents that will further the knowledge and understanding of the many intricate biological systems. But it is YOU, the scientists who use your imaginations, skills and brilliance to make the most of our targeting technology.

For nearly sixteen years, we have listened to your suggestions, read with appreciation the amazing ways you have dissected systems with our products and attempted to provide new tools to further your research. As a small company, we don't have the large marketing budget of the larger research suppliers (One full-page ad in *Science* is over $8500!). We depend a great deal on the word-of-mouth from knowledgeable customers and, in particular, the product reference you include in your publications. When a scientist wants to know what product to use to achieve the results published, a quick scan of the Materials section will tell them how to get what they need to enhance their own research.

So, thank you. We admire your innovation. We appreciate your science. We look forward to the next exciting results you will publish. Let us know what we can do to be a greater help in your research.

Targeting Talk: Product Questions

by Dr. Douglas Lappi

Q: We have a question about two 192-IgG-SAP lots. According to your data sheet there is an approximate 4-fold difference in ED50 between your new lot and old lot. We also observed a clear difference in behavior between animals dosed with the new batch and the old one. It is thus obvious that the new lot needs to be diluted to achieve the same results, however, we are uncertain if this can be calculated just based on the ED50 values. Do you have any experience about dose-responses with the different lots in terms of size of lesion?

A: We don't have an exact correlation between *in vitro* and *in vivo* activity, unfortunately. We always state on the data sheet to check a new batch on a small number of animals. Actually, I see that we merely suggest that; we'll change it to be more explicit.

"There may be lot-to-lot variation in material; working dilutions must be determined by end user. If this is a new lot, you must assess the proper working dilution before beginning a full experimental protocol."

Q: Concerning "Hum-ZAP (IT-22)" and "Rat-ZAP (IT-26)" are they monovalent or bivalent to their target immunogulobulins?

A: The secondary conjugates Hum-ZAP and Rat-ZAP are, in fact, bivalent and so do have the theoretical possibility of causing internalization when the primary would not -- a false positive. In fact, we have never heard of this happening, mainly because the theoretical situation is difficult to put into practice - probably things get a little bulky on the cell surface.

Our idea is that the secondary conjugates are meant for large-scale screening in a very cost-effective manner, and upon identification of a positive, that primary antibody can be biotinylated and tested *in vivo* with streptavidin-ZAP. Streptavidin-ZAP can also cause oligomerization, but it's used at equimolar amounts to the primary antibody, so that may not happen to an appreciable amount. However, the best method is to have a primary immunotoxin constructed through custom synthesis, in which saporin is directly coupled to the targeting agent.

http://www.atsbio.com/catalog/customs/conjugates.html

Q: Saporin has been shown to enzymatically inhibit the function of the ribosome, which follows that protein synthesis is then inhibited. Inhibition of protein synthesis brings about "cell death" to my knowledge.

To detect "cell death" usually does not take a longer time to detect than "growth inhibition," I suppose. So what I would like to ask you is: "at least" how many hours will it take to detect "cell death" caused by saporin.

In your protocol, the recommended duration of assay is 72 hours. Does that duration contain much allowance? Of course, the duration must be dependent on the speed (or efficiency) of internalization of saporin, I understand. But once saporin is internalized, how many hours (or minutes) will it take to kill the target cell?

A: 72 hours is for the great majority of cell lines, but there are a very few that require 48 hours and a very few that require 96 hours (maybe 1 of each of the 100 or so that we've tried). The variation in time from 72 hours is not much on the shorter side, but is only limited by the few living cells proliferating on the longer side.

It is easy to see dead cells in the microscope, so you may want to visually check your cells at different times to verify that 72 hours is correct.

How many hours will it take after internalization to kill a cell? Quite a few, because there are several processes that need to occur: the enzyme must inactivate a sufficient number of ribosomes to inhibit protein synthesis, and then the cell has to stop living because of the turnover and loss of those proteins. That takes time.

You may find this paper from long ago quite interesting: Olsnes *et al., J Biol Chem*. 251(13):3985-3992, 1976 Jul 10. It concerns relatives of saporin, ricin and abrin, that have their own, probably more efficient, cell binding chains and are true toxins (whereas saporin has no binding chain and therefore no real toxicity on its own). Incredibly, protein synthesis inhibition at high doses is underway after 1 hour!

Please email ats@ATSbio.com to get answers to your questions about ATS products.

Deletion of NPY/AGRP and POMC Neurons in the Arcuate Nucleus by Leptin-Saporin Produces Hyperphagia, Obesity and Changes in Diurnal Feeding Patterns in Rats

(continued from page 1)

Binding of the Lep-SAP to the OB-Rb receptor is the mechanism for selective internalization of saporin, a ribosomal toxin that destroys the cell. The goals of our study were (1) to determine whether this new Lep-SAP conjugate is an effective and selective lesioning agent and (2) to use this targeted toxin to further examine the role played by Arc OB-Rb-expressing neurons in the actions of endogenous leptin.

A dose-response analysis was conducted to determine the dose, volume and injection sites for lesioning the Arc. One or two sites per side were injected. Lesions were analyzed by quantification of NPY/AGRP and POMC neurons in the Arc by immunohistochemistry (IHC) and real-time PCR. Results showed that bilateral injections of 57 ng in 50 nl per injection site at two sites in the sagittal plane produced a lesion that was confined to, but extended throughout, the Arc.

To evaluate the specificity of Lep-SAP for lesioning leptin receptor-expressing neurons *in vivo*, unilateral injections of Lep-SAP or control Blank-SAP (B-SAP) were made into the Arc in Sprague Dawley (S/D) rats and Zucker *fa/fa* fatty rats, which have a mutation on the extracellular domain of the leptin receptor. IHC staining was performed to detect hypothalamic neurons positive for AGRP and the POMC-derived peptide, α-melanocyte-stimulating hormone (α-MSH). Figure 1 shows that in S/D rats, numbers of α-MSH- and AGRP-positive cells were significantly decreased on the Lep-SAP injected side to 16-18% of the numbers found on the non-injected side. In contrast, in Zucker *fa/fa* rats, numbers of α-MSH and AGRP neurons did not differ on the injected versus the noninjected sides. The effective reduction of leptin-receptor expressing neurons in the Arc of S/D rats by Lep-SAP and the failure of the toxin to lesion these neurons in the Zucker *fa/fa* rats lacking a functional leptin receptor indicates that Lep-SAP is an effective lesioning agent and that its internalization is dependent on the Ob-Rb receptor.

To evaluate the effect of Arc Lep-SAP on food intake and body weight, Lep-SAP or B-SAP control was injected bilaterally into Arc. We found that Lep-SAP rats rapidly became hyperphagic and obese after the injection and maintained a level of intake that was 2-fold greater than that of the B-SAP rats (Fig. 2). Diurnal rhythms of food intake were also altered. Daytime feeding was significantly enhanced. Lep-SAP rats did not respond to central leptin administration, had elevated plasma levels of glucose, free fatty acids,

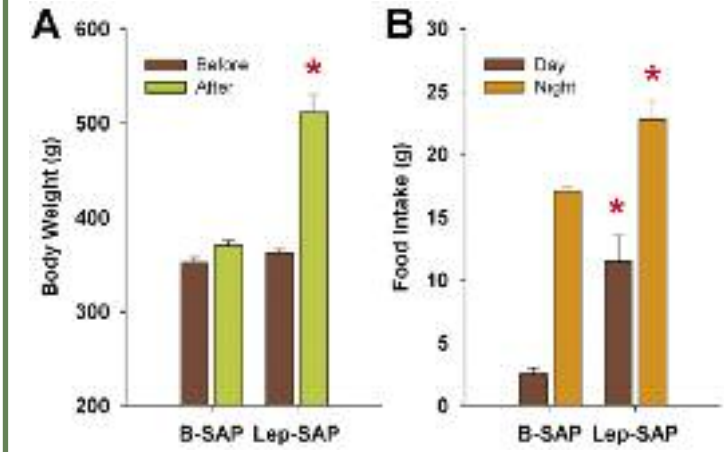

Figure 2. Body weight and food intake after bilateral Lep-SAP or B-SAP injection into the Arc. (A) Body weight at the time of and 4 weeks after Lep-SAP or B-SAP injections. (B) Food intake during the light phase and dark phase 12 days after Lep-SAP or B-SAP injection. *, $P < 0.01$; unpaired t-test vs. B-SAP rats.

triglycerides, insulin, and leptin, and had excessive fat deposits in liver, and in white and brown fat pads. Expression of clock-related genes was measured at a single time point in the light period by real-time PCR. Bmal1 expression in whole hypothalamus, liver, and white fat tissue of Lep-SAP rats was significantly decreased. Per1 expression was increased in liver and decreased in white fat tissue, compared to B-SAP controls. Per1 expression in the hypothalamus did not differ between Lep-SAP and B-SAP rats.

These results show that Lep-SAP effectively lesioned Arc NPY/AGRP and POMC neurons in a leptin receptor-dependent manner. In addition, these results support previous findings showing that Arc NPY/AGRP and POMC neurons play a critical role in the control of food intake and energy expenditure. Finally, we report new findings indicating a role for the Arc in the diurnal patterning of food intake and in central and peripheral clock-related gene expression. Thus, Lep-SAP is a useful new tool for studying the functions of leptin receptor-expressing neurons in specific brain sites.

Reference:

A.-J. LI, Q. WANG, T. T. DINH, S. RITTER, Leptin-saporin injection into the arcuate nucleus lesions NPY/AGRP and POMC neurons and produces hyperphagia, obesity and changes in diurnal feeding patterns in rats. Program No 374.5. 2009 Neuroscience Meeting Planner. Chicago, IL: *Society for Neuroscience*, 2009.

Am Assoc for Cancer Research
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Washington, DC
Booth #2011

Forum of European Neuroscience
July 3-7, 2010
Amsterdam, The Netherlands
Booth #507

Upcoming Events

Targeting Tools: Featured Products

Leptin-SAP

Leptin-SAP (Cat. #IT-47) is a conjugate between recombinant mouse leptin and saporin. Leptin is a 16 kDa protein hormone that activates leptin receptors, and plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin inhibits the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP), and increases the activity of neurons expressing α-melanocyte-stimulating hormone (α-MSH), and thereby is a very influential mediator of satiety. This new product could be used to eliminate leptin receptor-expressing cells as an excellent complement to NPY-SAP (IT-28) and Oxytocin-SAP (IT-46) in studying satiety, appetite, and metabolism. See cover article.

Distributor Highlight: South Korea

President and CEO of Sungwoo Life Science Co., Mr. Cho, visits with Denise Higgins and Dr. Douglas Lappi at the Society for Neuroscience meeting in Chicago.

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Hwang Sunghee is Technical Services Manager at Sunwoo Life Science and is ready to help our customers. We look forward to exciting research results from our new customers in South Korea.

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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.

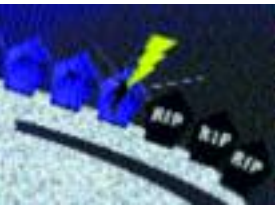
§ or anything recognized on the cell surface and internalized.

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 that 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 taken from the cover story, one letter to each block, to solve the puzzle.

PENLIT

TIAOGU

LITABLARE

TASLIGAT

GREENY

What the scientist discovered when he used only biodegradable reagents.

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

ANSWER: It was . . .

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by February 28, 2010.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 4.

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Apr-May-Jun 2010
Volume 11, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Depletion of syndecan-4$^+$ T lymphocytes by saporin-conjugated DC-HIL alleviates T cell-mediated inflammatory disease

Contributed by Kiyoshi Ariizumi, Hideo Akiyoshi, Jin-Sung Chung, Mizuki Tomiharu, Ponciano D. Cruz Jr.
Department of Dermatology, The University of Texas Southwestern Medical Center and Dermatology Section (Medical Service), Dallas Veterans Affairs Medical Center, Dallas, TX

T lymphocyte activation is regulated by stimulatory and inhibitory signals transduced by binding of T cell receptors to corresponding ligands on antigen-presenting cells (APC). Stimulatory receptors tend to be present constitutively even on resting T cells, whereas many inhibitory receptors require activation for expression.[1] Thus, inhibitory receptors may serve as a marker for the functional state of T cells.

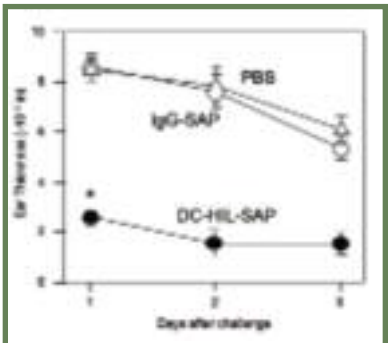

Fig. 1. Mice were injected i.v. with PBS, IgG-SAP, or DC-HIL-SAP (40 nM) 3 h prior to the challenge. Daily change in ear thickness was plotted for each panel.

We discovered a novel inhibitory pathway composed of the APC receptor DC-HIL and its exclusive T cell ligand, syndecan-4 (SD-4). DC-HIL specifically recognizes particular structures of heparan sulfate on SD-4 peculiar to T cells. SD-4 is expressed by activated (but not resting) T cells, including effector/memory CD4$^+$ and CD8$^+$ T cells. Infusion of soluble DC-HIL into mice inhibits the DC-HIL/SD-4 pathway, and results in enhanced immune responses. The current report addresses the hypothesis that depleting SD-4$^+$ T lymphocytes using DC-HIL conjugated to a toxin will suppress elicitation of a T cell-mediated inflammatory response.

We biotinylated and conjugated soluble DC-HIL receptor or control Fc alone (IgG-SAP) to Streptavidin-ZAP (streptavidin conjugated to saporin; Cat. #IT-27), and showed that DC-HIL-SAP binds specifically to activated T cells, is internalized by these cells, and inhibits T cell proliferation in a SD-4-specific manner. These results document that DC-HIL-SAP selectively kills SD-4$^+$ activated T cells.

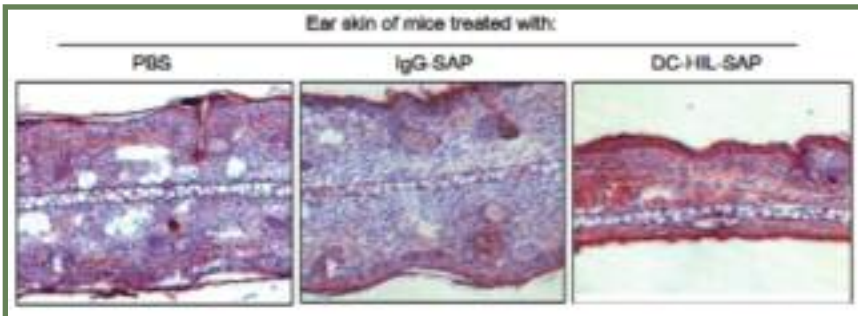

Fig. 2. On day 2 after challenge with oxazolone (Ox), ear skin specimens of mouse representative in each group were stained with hematoxylin and eosin, and histologically examined under magnification of 10 X.

We next examined the effect of DC-HIL-SAP on an ongoing contact hypersensitivity (CH) response, which is an established model of a delayed T cell-mediated response. Mice were sensitized to a contact allergen oxazolone (Ox) on abdominal skin (day 0), then challenged with Ox on ear skin (day 6). Mice were injected i.v. with DC-HIL-SAP, IgG-SAP (control conjugate), or PBS 3 h prior to challenge (Fig. 1). PBS-injected

(continued on page 6)

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Fab-ZAP line (see page 7)
Anti-6 His-ZAP (Cat. #IT-52; see page 7)
Anti-GFP-ZAP (Cat. #IT-53; see page 7)

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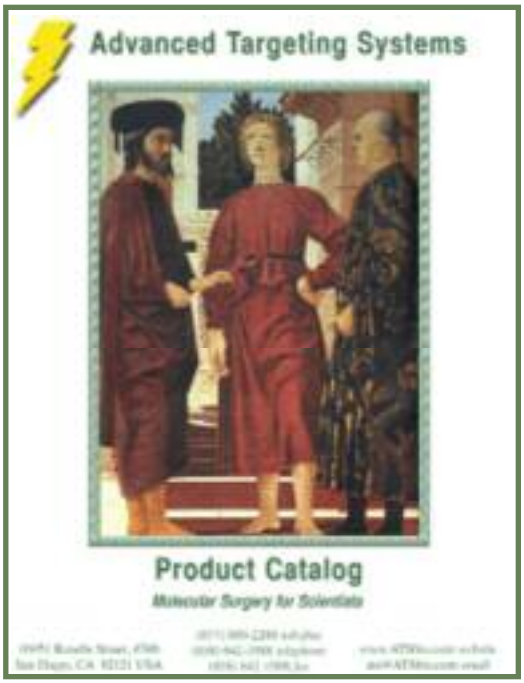

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Targeting Teaser Winners

The solution to the puzzle was:

Jumbles:

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AGOUTI
BILATERAL
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Answer: It was... EASY TO BE GREEN

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Solve the Teaser online at: www.ATSbio.com/news/10q2_teaser.html

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Enrichment of xenograft-competent genetically modified pig cells using a targeted toxin, isolectin BS-I-B4 conjugate

Akasaka E, Watanabe S, Himaki T, Ohtsuka M, Yoshida M, Miyoshi K, Sato M
Xenotransplantation 17(1):81-89, 2010.

Genetically-modified pigs lacking the galα1-3gal epitope may be suitable for production of organs that could be transplanted to humans. The ability to select for a homozygous population of donor somatic cells would accelerate the process of generating these animals, which would otherwise take approximately two years. The authors incubated a heterozygous population of 10^7 porcine embryonic fibroblasts with 1.6 µg of IB4-SAP (Cat. #IT-10). Even after six months the treated cells were negative for the αgal epitope.

Severe scene learning impairment, but intact recognition memory, after cholinergic depletion of inferotemporal cortex followed by fornix transection

Browning PG, Gaffan D, Croxson PL, Baxter MG
Cereb Cortex 20(2):282-293, 2010.

In order to directly test depletion of cholinergic neurons in the inferotemporal cortex on learning and memory the authors lesioned the inferotemporal cortex, the rostral entorhinal cortex, and the perirhinal cortex of monkeys with ME20.4-SAP (Cat. #IT-15), 56-64 injections of 0.02 μg per injection). The data suggest that episodic memory is in part controlled by interactions between the fornix and cholinergic input to the inferotemporal cortex.

Unique Contributions of Distinct Cholinergic Projections to Motor Cortical Plasticity and Learning

Conner JM, Kulczycki M, Tuszynski MH
Cereb Cortex [Epub Feb 24], 2010.

This work mapped the basal cholinergic forebrain system associations with skilled motor learning and motor function recovery after cortical injury. Rats were lesioned with 192-IgG-SAP (Cat. #IT-01). The animals received either two rostrocaudal injections of 75-112 ng; two 19-ng injections into the "prefrontal depletion site"; or two 19-ng injections into the "motor cortex depletion site." Loss of motor cortex cholinergic systems disrupts map plasticity and skilled motor behavior, indicating that control of these systems rests within the motor cortex.

Depleting Syndecan-4+ T Lymphocytes Using Toxin-Bearing Dendritic Cell-Associated Heparan Sulfate Proteoglycan-Dependent Integrin Ligand: A New Opportunity for Treating Activated T Cell-Driven Disease

Akiyoshi H, Chung JS, Tomihari M, Cruz PD, Jr., Ariizumi K
J Immunol 184(7):3554-3561, 2010.

The dendritic cell-associated heparin sulfate proteoglycan-dependent integrin ligand (DC-HIL) exclusively associates with syndecan-4 (SD-4), which is expressed on some activated T-cells. The authors biotinylated DC-HIL and combined it with streptavidin-ZAP (Cat. #IT-27) and used in culture at a concentration of 10 µg/ml. Only activated T cells were bound and eliminated.
(See cover article.)

Transplant of GABAergic precursors restores hippocampal inhibitory function in a mouse model of seizure susceptibility

Zipancic I, Calcagnotto ME, Piquer-Gil M, Mello LE, Alvarez-Dolado M
Cell Transplant [Epub Feb 8], 2010.

Although medial ganglionic eminence-derived cells can be grafted into the neonatal brain and become functionally mature GABAergic neurons, it is not clear whether the grafted cells can rescue loss of function. The authors injected mice with 1.6-2.0 ng of SSP-SAP (Cat. #IT-11) into the anterior and posterior hippocampus to eliminate GABAergic interneurons. Neuron function in mice receiving the grafts returned to near normal.

Arcuate nucleus destruction does not block food deprivation-induced increases in food foraging and hoarding

Dailey MJ, Bartness TJ
Brain Res [Epub Feb 4], 2010.

While some aspects of food intake are understood, mechanisms that control hoarding of food have not been identified. This work investigates the role of NPY in the arcuate nucleus (Arc) in hoarding. Siberian hamsters received 48-ng injections of NPY-SAP (Cat. #IT-28) into the Arc; Blank-SAP (Cat. #IT-21) was used as a control. In lesioned animals food deprivation-induced hoarding was increased 100%, but baseline foraging and food hoarding were unchanged.

Dorsal horn neurons expressing NK-1 receptors mediate scratching in rats

Carstens EE, Carstens MI, Simons CT, Jinks SL
Neuroreport 21(4):303-308, 2010.

The itch signal is passed through the superficial dorsal horn. The authors investigated whether ablation of NK-1 receptor-expressing neurons in this area would affect itch-related scratching behavior. Rats received 20 µl of 2.27-µM SP-SAP as an intracisternal injection. The reduction in itch response to intradermal 5-hydroxytryptamine indicates that NK-1 receptor-expressing superficial dorsal horn neurons are important for spinal itch transmission.

Toxin-Coupled MHC Class I Tetramers Can Specifically Ablate Autoreactive CD8+ T Cells and Delay Diabetes in Nonobese Diabetic Mice

Vincent BG, Young EF, Buntzman AS, Stevens R, Kepler TB, Tisch RM, Frelinger JA, Hess PR
J Immunol [Epub Mar 10], 2010.

MHC class I tetramers have been used to identify antigen-specific cells. In this work the authors used a biotinylated tetramer in conjunction with streptavidin-ZAP (Cat. #IT-27) to eliminate a specific subset of reactive

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

T cells associated with islets *in vivo*. NOD mice received three 4.36-µg intravenous injections of the tetramer/saporin complex over 12 days. The onset of type I diabetes in the treated mice was significantly delayed.

An early sympathetic nervous system influence exacerbates collagen-induced arthritis via CD4+CD25+ cells

Harle P, Pongratz G, Albrecht J, Tarner IH, Straub RH
Arthritis Rheum 58(8):2347-2355, 2008.

The sympathetic nervous system can play conflicting roles in collagen-induced arthritis (CIA). CD4+CD25+ T cells can play an immunoregulatory effect in this system depending on the expression of the FoxP3 transcription factor. Mice received 5-µg ip injections of anti-DBH-SAP (Cat. #IT-03) to induce an early sympathectomy. The results indicate that the sympathetic nervous system increases disease severity in CIA by stimulating some of the proinflammatory aspects of CD4+CD25+ T cells.

An opposing time-dependent immune-modulating effect of the sympathetic nervous system conferred by altering the cytokine profile in the local lymph nodes and spleen of mice with type II collagen-induced arthritis

Harle P, Mobius D, Carr DJ, Scholmerich J, Straub RH
Arthritis Rheum 52(4):1305-1313, 2005.

In this work the authors examined the role of the sympathetic nervous system (SNS) in late stages of chronic arthritis. 5-µg intraperitoneal injections of anti-DBH-SAP (Cat. #IT-03) were given in mice. The results demonstrate that the SNS supports inflammation during the asymptomatic phase of arthritis, but inhibits inflammation during the chronic symptomatic phase.

The brainstem noradrenergic systems in stress, anxiety, and depression

Itoi K, Sugimoto N
J Neuroendocrinol [Epub Feb 20], 2010.

In this review the authors examine the relationship between the central noradrenergic system, fear/anxiety states, and depression. The use of anti-DBH-SAP (Cat. #IT-03) to investigate the function of the noradrenergic system in these paradigms is described.

Evaluation of side effects through selective ablation of the mu opioid receptor expressing descending nociceptive facilitatory neurons in the rostral ventromedial medulla with dermorphin-saporin

Cao F, Chen SS, Yan XF, Xiao XP, Liu XJ, Yang SB, Xu AJ, Gao F, Yang H, Chen ZJ, Tian YK
Neurotoxicology 30(6):1096-1106, 2009.

It has been shown that injection of dermorphin-SAP (Cat. #IT-12) into the rostral ventromedial medulla (RVM) can abolish descending facilitation. In this work side effects produced by a 3-pmol injection of dermorphin-SAP into the RVM of rats were assessed (Saporin, Cat. #PR-01, was used as a control). Following select physiological functions over a three-month period post-lesion demonstrated that treatment with this targeted toxin produces no long-standing adverse toxicity.

Cardiovascular and behavioural responses to conditioned fear and restraint are not affected by retrograde lesions of A5 and C1 bulbospinal neurons

Vianna DM, Carrive P
Neuroscience 166(4):1210-1218, 2010.

To investigate the role of A5 neurons in some forms of psychological stress the authors injected 22 or 44 ng of anti-DBH-SAP (Cat. #IT-03) into the spinal cord of rats. Mouse IgG-SAP (Cat. #IT-18) was used as control. The data show that A5 presympathetic neurons are not essential for the expression of the tachycardic and pressor responses to conditioned fear and restraint.

Distinct Neural Pathways Mediate {alpha}7 Nicotinic Acetylcholine Receptor-Dependent Activation of the Forebrain

Thomsen MS, Hay-Schmidt A, Hansen HH, Mikkelsen JD
Cereb Cortex [Epub Jan 4], 2010.

α7 nicotinic acetylcholine receptor (nAChR) agonists are potential treatments for some aspects of schizophrenia. The authors examine whether cholinergic neurons in the horizontal limb of the diagonal band of Broca (HDB) are a target for this treatment. Rats received 300-ng injections of 192-IgG-SAP (Cat. #IT-01) into the HDB. The results demonstrate that cholinergic neurons in the HDB are essential for α7 nAChR agonist activation of the medial prefrontal cortex.

Targeted Ablation of Cardiac Sympathetic Neurons Reduces the Susceptibility to Ischemia-Induced Sustained Ventricular Tachycardia in Conscious Rats

Lujan HL, Palani G, Zhang L, Dicarlo SE
Am J Physiol Heart Circ Physiol [Epub Feb 19], 2010.

Reduction of cardiac sympathetic activity protects against ventricular tachy-arrhythmias, which are the leading cause of death in industrially-developed countries. Rats received 10-µg injections of CTB-SAP (Cat. #IT-14) into each stellate ganglion. Using comparison of ventricular tachycardia onset times after coronary artery occlusion it was found that lesioned rats were less susceptible to tachycardia events.

The hyperalgesic effects induced by the injection of angiotensin II into the caudal ventrolateral medulla are mediated by the pontine A(5) noradrenergic cell group

Marques-Lopes J, Pinho D, Albino-Teixeira A, Tavares I
Brain Res [Epub Feb 19], 2010.

Injection of angiotensin II into the caudal ventrolateral medulla (CVLM) has been shown to induce angiotensin type 1 receptor-mediated hyperalgesia. Here the authors lesioned the pontine A5 cell group with anti-DBH-SAP (Cat. #IT-03) to evaluate the role of these neurons in this model. Rats received a 1.1-µg injection of anti-DBH-SAP into the

(continued on page 5)

Targeting Talk: Product Questions

by Dr. Douglas Lappi

Q: Our lab has been working with Orexin-SAP (Cat. #IT-20) and we need to order more to complete our experiments. Unfortunately, your website says the product is discontinued. Could you let us know why you have stopped selling Orexin-SAP? Is it possible for us to get more of it in the future?

A: Thank you for your inquiry regarding Orexin-SAP. We have been working for several months to try to produce a new lot of this targeted toxin. We discontinued the sale of this targeted toxin because we could not validate the product. We have a Quality Control (QC) assay, but the material we prepared did not perform to the level of the previous Orexin-SAP that we have been selling for several years.

Peptides made by three different suppliers have been conjugated; none performed as the previous lot of Orexin-SAP did. We don't have an explanation for why the new material doesn't work in our *in vitro* QC assay. We decided it was best to discontinue distribution of Orexin-SAP.

Since announcing the decision to discontinue Orexin-SAP, we have heard from several scientists, such as yourself, expressing the need for more of this targeted toxin. ATS is proud of our reputation for quality targeting reagents, and we are reluctant to promote a product for which we cannot provide QC data.

So, we came up with a solution. We do not currently have *in vitro* or *in vivo* data for any of the new lots of Orexin-SAP, and are entertaining proposals for a collaboration with experienced researchers who will test these lots and share their data with us. Collaborators will receive samples from 2-4 different lots of Orexin-SAP, and aliquots of Blank-SAP (control conjugate) will be provided at no charge.

Contact Denise Higgins (ats@ATSbio.com) if you are interested in this opportunity. We look forward to working with you.

Targeting Topics: Recent Scientific References

(continued from page 4

CVLM. Behavioral responses indicate that loss of noradrenergic neurons in the CVLM partially prevented angiotensin II-induced hyperalgesia.

Serotonin transport and metabolism in the mammary gland modulates secretory activation and involution

Marshall AM, Nommsen-Rivers LA, Hernandez LL, Dewey KG, Chantry CJ, Gregerson KA, Horseman ND
J Clin Endocrinol Metab 95(2):837-846, 2010.

This work begins to examine the role of the serotonin reuptake transporter (SERT) in the regulation of lactation homeostasis. The SERT monoclonal antibody (Cat. #AB-N09) was used for immunohistochemistry.

The cerebellum harbors a circadian oscillator involved in food anticipation

Mendoza J, Pevet P, Felder-Schmittbuhl MP, Bailly Y, Challet E
J Neurosci 30(5):1894-1904, 2010.

The authors report on a circadian oscillator in the cerebellum that is sensitive to feeding cues. Mice received icv injections of 0.12, 0.25, or 0.50 µg of OX7-SAP (Cat. #IT-02). Lesioned animals displayed attenuated food-anticipatory activity, and less locomotor activity after fasting.

Does Age Matter? Behavioral and Neuro-anatomical Effects of Neonatal and Adult Basal Forebrain Cholinergic Lesions

De Bartolo P, Cutuli D, Ricceri L, Gelfo F, Foti F, Laricchiuta D, Scattoni ML, Calamandrei G, Petrosini L
J Alzheimers Dis [Epub Feb 17], 2010.

The authors characterized the differences caused by age on the effect of cholinergic lesions of the basal forebrain. Seven-day-old rats received 210-ng bilateral icv injections of 192-IgG-SAP (Cat. #IT-01). Eighty-day-old rats received 4-µg bilateral icv injections. Both experimental groups displayed similar behavior, indicating that development of a depleted cholinergic system yields similar results to cholinergic dysfunction in adulthood.

Recent Progress in Research on Ribosome Inactivating Proteins

Ng TB, Wong JH, Wang H
Curr Protein Pept Sci [Epub Dec 1], 2009.

Brief descriptions of research done using 192-IgG-SAP (Cat. #IT-01), OX7-SAP (Cat. #IT-02), dermorphin-SAP (Cat. #IT-12), anti-SERT-SAP (Cat. #IT-23), SSP-SAP (Cat. #IT-11), anti-DBH-SAP (Cat. #IT-03), CTB-SAP (Cat. #IT-14), and other conjugates are provided.

Methylphenidate-induced impulsivity: pharmacological antagonism by beta-adrenoreceptor blockade

Milstein JA, Dalley JW, Robbins TW
J Psychopharmacol 24(3):309-321, 2010.

In this work bilateral 20-ng intracortical injections of anti-DBH-SAP (Cat. #IT-03) were used to examine the role of noradrenergic neurons in the control of psychostimulant-induced impulsivity. Although β-adrenoreceptor blockade abolished this impulsivity, lesioning noradrenergic neurons in the cortex had no effect.

Depletion of syndecan-4$^+$ T lymphocytes by saporin-conjugated DC-HIL alleviates T cell-mediated inflammatory disease

(continued from page 1)

mice developed strong ear swelling, whereas DC-HIL-SAP-injected mice exhibited markedly reduced ear swelling by 80%. IgG-SAP had no effect. Our DC-HIL-SAP concentration was optimal since 20 nM caused 50% suppression, whereas 80 nM produced 80% reduction (similar dose of IgG-SAP causing increased toxicity). Histologic examination of Ox-painted ear skin in DC-HIL-SAP-injected mice revealed less thick ears and fewer infiltrating leukocytes (Fig. 2). Injection of DC-HIL-SAP following Ox challenge also reduced CH response. The unresponsive state to Ox lasted for 3 weeks (Fig. 3), even as these same mice were able to mount effective CH response against another contact allergen 2,4,6-trinitrochlorobenzene (TNCB) (Fig. 4).

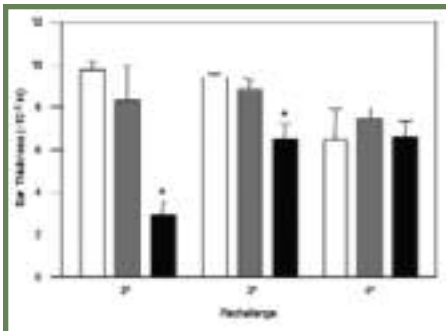

Fig. 3. These mice were kept for one week and then rechallenged with Ox weekly for second (2º), third (3º) and fourth challenges (4º). Ear thickness was measured one day following challenge. *p<0.001 and **p=0.003: Student's t test vs. ear thickness treated with IgG-SAP.

These results indicate that a single infusion of DC-HIL-SAP efficiently blocks elicitation of an established immune response that lasts for 3 weeks and is restricted to the antigen introduced at the time of treatment.

We also examined the ability of DC-HIL-SAP to deplete SD-4$^+$ T cells in immunized mice. Two days after challenging sensitized mice treated with DC-HIL-SAP or controls, SD-4$^+$ T cells in Ox-painted ear skin or in draining lymph nodes (DLN) were counted by immunofluorescent staining (Fig. 5A) or by flow cytometry (Fig. 5B), respectively. There were none-to-very few T cells in untreated skin, but many CD4$^+$ and CD8$^+$ T cells in Ox-painted skin, almost all of which were SD-4$^+$ (Fig. 5A). Numbers of CD4$^+$ and CD8$^+$ T cells in skin of mice injected with IgG-SAP were similar to those of mice treated with PBS, whereas both were reduced markedly following DC-HIL-SAP infusion. In DLN, infusion of DC-HIL-SAP depleted by 40% CD4$^+$ and CD8$^+$ T cells. These results indicate that a single infusion of DC-HIL-SAP depletes SD-4$^+$ T cells in the inflamed skin and DLN.

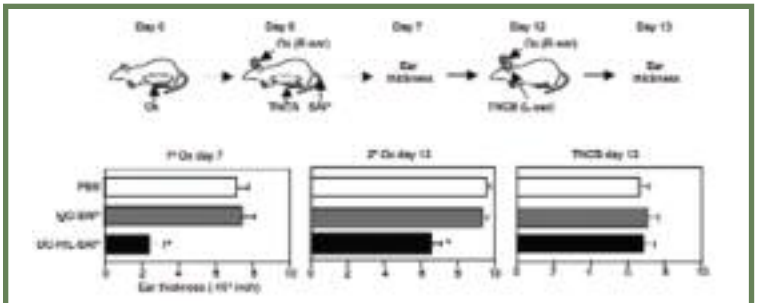

Fig. 4. BALB/c mice (n=4) were sensitized with Ox on day 0, i.v. injected with PBS or SAP conjugate 3 h prior to challenge (day 6). On the same day, mice were challenged with Ox and solvent alone on right (R-ear) and left ears (L-ear), respectively, and also sensitized to TNCB. On day 7, ear thickness was measured (1º Ox challenge). Day 12, all mice were challenged with Ox (2º Ox challenge) and TNCB on right and left ears, respectively. Ear thickness shown is measured on day 1 after every challenge. *p<0.05; as compared with ear thickness treated with IgG-SAP.

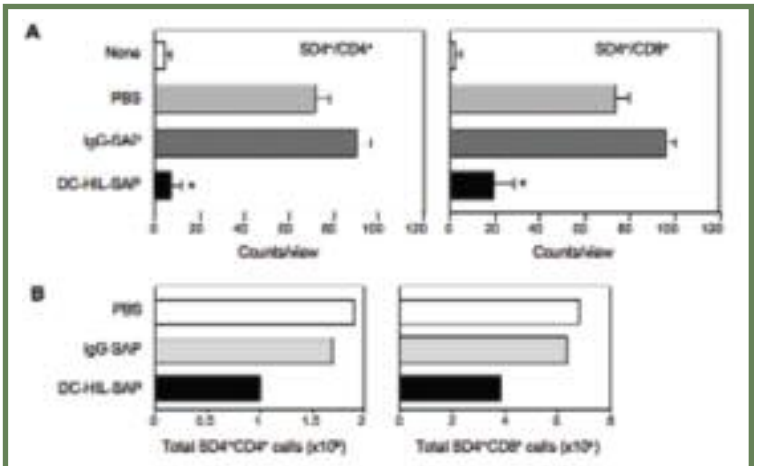

Fig. 5. Infusion of DC-HIL-SAP depletes SD-4+ T cells efficiently in Ox-painted skin and draining lymph nodes (DLN). BALB/c mice (n=3) were sensitized, injected with PBS or SAP conjugate, and challenged. Two days after challenge, ear skin (A) or DLN (B) were procured, doubly-stained with anti-CD4 or CD8 Ab and anti-SD-4 Ab. **(A)** Using confocal microscopy, SD-4$^+$/CD4$^+$ or SD-4$^+$/CD8$^+$ T cells were counted in three separate views, and the average with standard deviation is shown graphically. * p<0.01 as compared with % in skin of mice infused with IgG-SAP. **(B)** CD4$^+$ or CD8$^+$ T cells were purified from pooled DLN cells and counted by flow cytometry for SD-4$^+$/CD4$^+$ or SD-4$^+$/CD8$^+$ T cells per DLN.

Our studies in mice indicate that SD-4 can be targeted using toxin-bearing DC-HIL to alleviate a cutaneous inflammatory response that may find applications in many human disease states. The targeted nature (SD-4$^+$ T cells) of this treatment may hold special advantage with respect to safety.

References/Footnotes:

1. T cell expression profiles of these receptors overlap but are disparate; cytotoxic T Lymphocyte antigen-4 (expressed by almost all recently activated T cells), programmed cell death-1 (restricted to effector T cells), B and T lymphocyte attenuator and T cell immunoglobulin mucin 3 (expressed preferentially by Th1 cells). Moreover, sustained high-level of programmed cell death-1 expression is a marker for T cells undergoing exhaustion in chronic viral infections and in cancer.

2. Chung J-S, Sato K, Dougherty I, Cruz PD Jr, Ariizumi K. DC-HIL is a negative regulator of T cell activation. *Blood* 109:4320-4327, 2007.

3. Akiyoshi H, Chung J-S, Tomihari M, Cruz PD Jr, Ariizumi K. Depleting syndecan-4+ T lymphocytes using toxin-bearing DC-HIL: A new opportunity for treating activated T cell-driven disease. *J Immunol* April 2010.

Targeting Tools: Featured Products

New Secondary Conjugates -- Fab-ZAPs -- Use Monovalent Secondary Antibodies Linked to Saporin

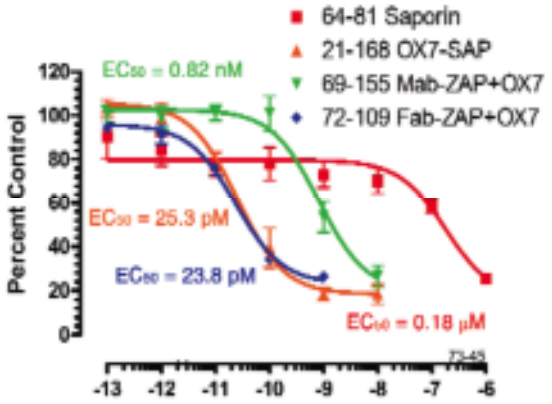

Fig. 1. PC12 cells were plated at 5000 cells/90 µl/well and incubated overnight. Saporin (PR-01) and OX7-SAP (IT-02) dilutions were made in cell media, and 10 µl was added to each well. OX7 antibody (AB-N08) was diluted in cell media containing, at a final concentration, either 100 ng/10 µl Mab-ZAP or 45 ng/10 µl Fab-ZAP, and 10 µl was added to each well. The plates were incubated 72 hours. The medium was dumped off of the plate, and the cells were fixed with ice-cold 10% TCA for 1 hr at 4°C. The plate was washed three times with tap water and allowed to air dry. 50 µl of 0.4% sulfarhodamine B/1% acetic acid was added to each well and the plate was incubated for 30 min at room temperature. The plate was washed three times with 1% acetic acid and allowed to air dry. The dye was solubilized with 100 µl of 10 mM unbuffered tris base per well, with 5 min of gentle shaking. The plate was read at 564 nm, and data analysis was done with Prism software (GraphPad, San Diego).

For the past decade scientists have extensively used secondary conjugates (e.g. Mab-ZAP; Cat. #IT-04, and Hum-ZAP; Cat. #IT-22) to make their own targeted toxins for *in vitro* use. The ability to combine: 1) a primary antibody to a cell-surface marker with 2) a secondary conjugate, in order to eliminate cells is a valuable tool in determining antibody specificity and internalization.

In theory, there are some possible limitations to the original product line of secondary conjugates to serve as a singular diagnostic tool. These secondary conjugates are made with whole molecule IgG secondary antibodies that recognize both the heavy and light chain of primary antibodies. The bivalent nature of these antibodies offers the possibility that cross-linking could occur on the cell surface, which can contribute to a phenomenon known as "cap formation." When molecules on the surface of a cell are cross-linked they are moved to one end of the cell to form a "cap," the formation of which can induce some level of endocytosis that leads to false positives (due to inappropriate internalization) in a cytotoxicity assay.

Although we have no data or customer feedback that demonstrates that this has actually occurred in practice, we decided to be proactive and provide our customers with additional tools to meet any potential concerns. As a result, ATS is proud to release a new line of secondary conjugates (Fab-ZAP) produced with monovalent antibodies. These products eliminate the possibility of cap formation as cross-linking of the Fab-ZAP molecules cannot occur, while preserving all of the qualities that make an effective *in vitro* diagnostic tool. The Fab-ZAP products will still recognize the heavy and light chains of antibodies, and should be used in the same way and at the same molar concentrations as the original secondary conjugates. In fact, preliminary assays using Fab-ZAP-mouse have demonstrated an unexpected lower EC_{50} when directly compared to Mab-ZAP in a cytotoxicity assay (see Fig. 1).

Fab-ZAP (mouse) - Cat. #IT-48, 25 µg / 100 µg / 250 µg
Fab-ZAP (mouse) uses your primary mouse monoclonal IgG antibody to target and eliminate cells that recognize your primary antibody.
Fab-ZAP (human) and Fab-ZAP (rabbit) - *In Production*

Anti-6 His-ZAP (Cat. #IT-52)

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§ or anything recognized on the cell surface and internalized.

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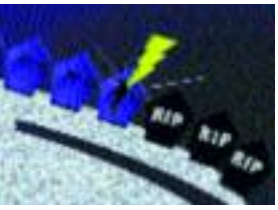

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles taken from the cover story, one letter to each block, to solve the puzzle.

CANDYNES

INDAVERTPITS

SKLOBC

UCSTOUNEA

YEWKEL

What the scientists did before they began their experiment.

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

ANSWER: Got their . . . ☐☐☐☐☐ ☐☐ ☐ ☐☐☐

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by May 31, 2010.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 4.

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Jul-Aug-Sep 2010
Volume 11, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Role of Cell Fate Determinants in a Model of Spinal Cord Neurotoxic Lesion Induced by Cholera Toxin B-Saporin

Contributed by Rosario Gulino, Vincenzo Perciavalle and Massimo Gulisano
Dept of Physiological Sciences, Univ of Catania, Viale Andrea Doria, 6 - I95125 Catania ITALY

A promising approach for central nervous system (CNS) repair consists in the activation of endogenous neural precursor cells (NPCs), but this process is less efficient in the spinal cord (SC) following a spinal cord injury (SCI). Another process promoting a functional restoration after SCI consists in the reorganization of spared pathways by mechanisms involving the modulation of synaptic efficacy.[1,2] Sonic hedgehog (Shh), Notch-1 and Numb are involved in the stem cell functioning[3] and, additionally, Notch-1 has a role as modulator of synaptic plasticity.[4,5] However, little is known about the role of these proteins in the adult SC after removal of motoneurons.

In this study, we injected Cholera toxin-B saporin (CTB-SAP, Cat. #IT-14) into the gastrocnemius muscle to induce a mild depletion (about 30%; Fig. 1) of motoneurons within the lumbar SC of adult mice and analyzed the expression of Choline acetyltransferase (ChAT), Synapsin-I, Shh, Notch-1 and Numb proteins, by western blotting. The functional outcome of the lesion, as well as the possible recovery of locomotion, were monitored by grid walk and rotarod tests.

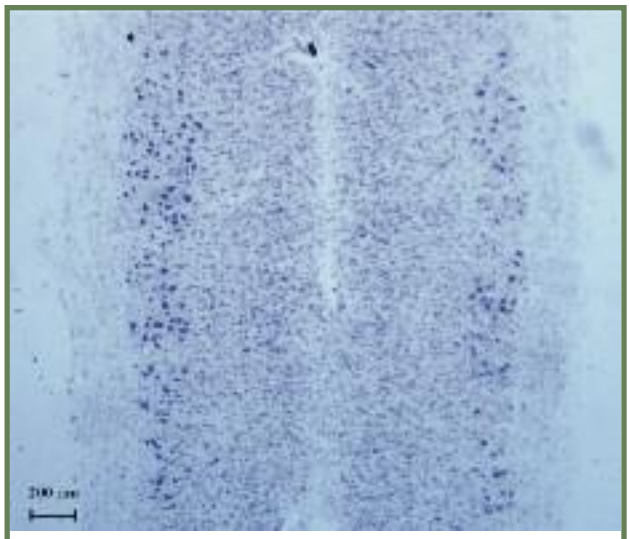


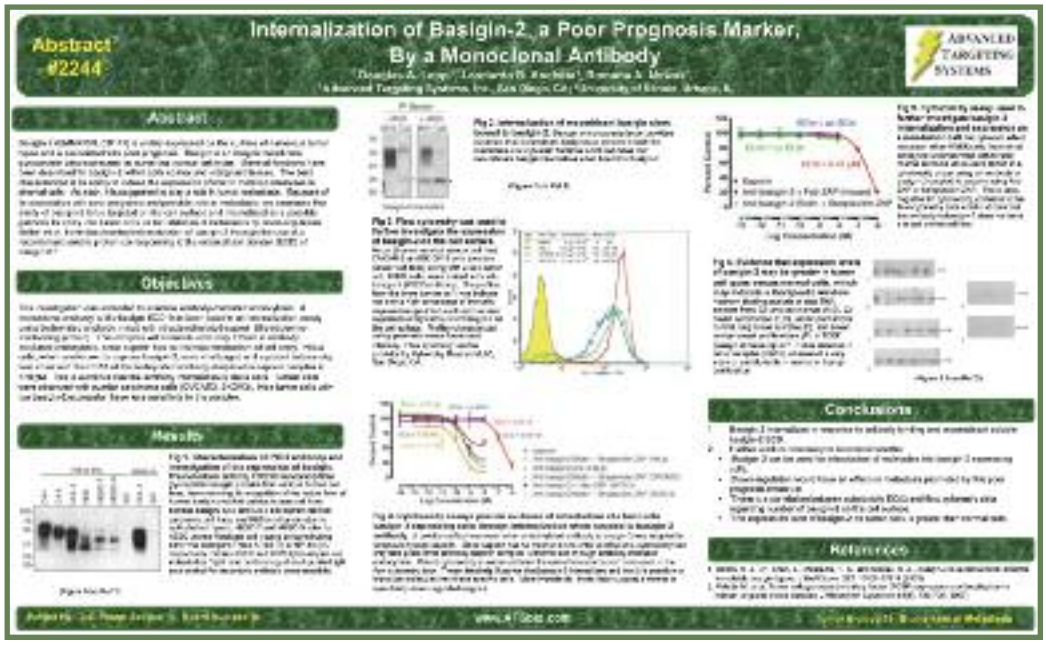
Fig. 1. Effect of CTB-SAP on the number of surviving motoneurons in the lumbar SC, one week after lesion, as observed in cresyl violet-stained SC sections obtained from unilaterally injected animals (n=5). The same results were found one month after lesion. The original picture has been adjusted in brightness and contrast.

We observed that the motoneuron depletion was paralleled by a worsening of functional performances and by the down-regulation of expression of ChAT (27%), Synapsin-I (33%), Numb (32%) and Shh (12%) at one week after the lesion. Notch-1 appeared not significantly affected (Fig. 2). A significant recovery of both functional performance and protein expression levels was seen at one month after the lesion (Fig. 2). We next observed that the performance of mice at grid walk and rotarod tests strongly correlates with the expression levels of ChAT and Numb proteins in the lesioned but not in the control animals (Fig. 3). Moreover, the expression levels of ChAT and Synapsin-I, which can be considered as markers of synaptic function and plasticity, were found associated to the levels of Shh and Notch-1 (Fig. 4). Notably, the expression of the mentioned proteins has been observed in the neuronal cells, whereas glial cells appeared negative (Fig. 2). Together, these results suggest that the spontaneous recovery of locomotion could be

(continued on page 6)

Poster at American Association for Cancer Research Meeting

At this year's AACR meeting, Leonardo Ancheta, scientist and Product Manager from ATS, presented a poster on our new monoclonal antibody to Basigin-2 (Cat. #AB-42). The poster describes our work with the antibody and the immunotoxin made from it (Cat. #IT-54). Basigin-2 (EMMPRIN, CD147) is widely expressed on the surface of numerous tumor types and is associated with poor prognosis. Because of its association with poor prognosis and possible role in metastasis, we examined the ability of basigin-2 to be targeted on the cell surface and internalized as a possible gateway for entry into tumor cells, or for inhibition of metastasis by down-regulation.



Our study reached the following conclusions: 1) Basigin-2 internalizes in response to antibody binding and recombinant soluble basigin-2 ECD. 2) Further work is necessary to determine whether a) Basigin-2 can be used for introduction of molecules into basigin-2 expressing cells, b) Down-regulation would have an effect on metastasis promoted by this poor prognosis molecule, c) There is a correlation between cytotoxicity EC_{50} and flow cytometry data regarding number of basigin-2 on the cell surface, and d) The expression level of basigin-2 on tumor cells is greater than normal cells.

ATS will be continuing its research with basigin-2 with a SBIR Phase I grant from the National Cancer Institute. The purpose of this Phase I proposal is to provide strong evidence that Anti-Basigin2-SAP is able to eliminate tumor cells that are highly expressing basigin-2. This will provide a rationale to perform *in vivo* work in mouse tumor models in Phase II to determine anti-tumor activity and to begin design of the molecule as a meaningful systemic drug for treatment of tumors with high metastatic potential.

Upcoming Events

FENS
(Forum of European Neuroscience)
July 3-7, 2010 • Booth #507
Amsterdam, The Netherlands

Society for Neuroscience
Nov 14-17 • Booth #3525
San Diego, CA

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles:

SYNDECAN
STREPTAVIDIN
BLOCKS
CUTANEOUS
WEEKLY

Answer: Got their... DUCKS IN A ROW

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

WINNERS: Barbara Attardi, Bioqual, Inc., Rockville, MD * Seto Chice, SUNY-HSC, Brooklyn, NY * Glenn Kageyama, Cal Poly Pomona Univ, Pomona, CA * Karyn DiNovo, Midwestern Univ, Downers Grove, IL * E Polin-Purch, Montefiore Hospital, Bronx, NY * April Price, UCSF, San Francisco, CA

Solve the Teaser online at: www.ATSbio.com/news/10q3_teaser.html

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Hindbrain Catecholamine Neurons Modulate the Growth Hormone But Not the Feeding Response to Ghrelin

Emanuel AJ, Ritter S
Endocrinology Epub, 2010.

In this work the authors investigated the role of hindbrain catecholamine neurons in the response to a gastrointestinal peptide, ghrelin. Rats received 42-ng injections of anti-DBH-SAP (Cat. #IT-03) into the paraventricular nucleus of the hypothalamus. Saporin (Cat. #PR-01) was used as a control. Lesioned animals had a prolonged growth hormone (GH) response to ghrelin administration as compared to controls, but the feeding response was unchanged. The results indicate that ghrelin or GH may be involved with a negative feedback response controlling GH levels.

BMP9 (bone morphogenetic protein 9) induces NGF as an autocrine/paracrine cholinergic trophic factor in developing basal forebrain neurons

Schnitzler AC, Mellott TJ, Lopez-Coviella I, Tallini YN, Kotlikoff MI, Follettie MT, Blusztajn JK
J Neurosci 30(24):8221-8228, 2010.

Bone morphogenetic protein (BMP) 9 is a cholinergic differentiation factor that increases acetylcholine synthesis and choline acetyltransferase gene expression. The authors investigated whether BMP9 could induce cholinergic trophic factors in murine septal cells. One experiment involved the sorting of E18 septal cells using anti-p75 (Cat. #AB-N01AP, 5 µg/2 x 10^6 cells). The increased NGF gene expression in response to BMP9 in p75-positive basal forebrain cholinergic neurons indicates an autocrine/paracrine role for NGF in the development and maintenance of these cells.

Hyperalgesic priming is restricted to isolectin B4-positive nociceptors

Joseph EK, Levine JD
Neuroscience Epub, 2010.

Hyperalgesic priming is an injury that induces a chronic pain state marked by the presence of inflammatory cytokines. The authors evaluated which populations of nociceptors are involved in the priming process. Rats that received 3.2-µg intrathecal injections of IB4-SAP (Cat. #IT-10) failed to establish priming. Acute mechanical hyperalgesia could still be induced, indicating that IB4+ nociceptors are necessary for priming, but a different nociceptor group is involved with nociceptor sensitization.

Expression of cell fate determinants and plastic changes after neurotoxic lesion of adult mice spinal cord by cholera toxin-B saporin

Gulino R, Perciavalle V, Gulisano M
Eur J Neurosci 31(8):1423-1434, 2010.

Sonic hedgehog, Notch-1, and Numb are proteins known to be involved in the function of stem cells. Understanding of how they might work in adults may provide methods to improve recovery from spinal cord injury. In this work the authors injected 3 µg of CTB-SAP (Cat. #IT-14) into the medial and lateral gastrocnemius muscles of mice. Analysis of protein levels following motoneuron depletion gives some insight into the molecular framework of nerve injury. (See cover article.)

Utilization of the least shrew as a rapid and selective screening model for the antiemetic potential and brain penetration of substance P and NK1 receptor antagonists

Darmani NA, Wang Y, Abad J, Ray AP, Thrush GR, Ramirez J
Brain Res 1214:58-72, 2008.

This work investigated the role of central tachykinin NK1 receptors in delayed phase vomiting caused by chemotherapeutics. Least shrews received 1.2 mg/kg intraperitoneal injections of SSP-SAP (Cat. #IT-11). Saporin (Cat. #PR-01) and blank-SAP (Cat. #IT-21) were used as controls. In response to administration of a NK1 receptor agonist, lesioned animals vomited less than the control group, indicating an important role for NK1 receptors in emesis.

Orexin mediates initiation of sexual behavior in sexually naive male rats, but is not critical for sexual performance

Di Sebastiano AR, Yong-Yow S, Wagner L, Lehman MN, Coolen LM
Horm Behav Epub, 2010.

In this work the role of endogenous orexin A and B in male sexual behavior was investigated. Rats received a total of 400 ng of orexin-SAP (discontinued) into the hypothalamus in each hemisphere. Blank-SAP (Cat. #IT-21) was used as a control. The lesions facilitated initiation of sexual behavior in naïve males, and reduced anxiety-like behaviors. The data suggest that orexin may play a role in arousal and anxiety related to sexual behavior in naïve animals, but is not critical for performance or motivation.

Selective Lesion of the Developing Central Noradrenergic System: Short- and Long-Term Effects and Reinnervation by Noradrenergic-Rich Tissue Grafts

Coradazzi M, Gulino R, Garozzo S, Leanza G
J Neurochem Epub, 2010.

The authors removed noradrenergic neurons in the locus coeruleus/subcoeruleus complex of neonatal rats with 0.25-1.0 µg bilateral injections of anti-DBH-SAP (Cat. #IT-03). No damage was seen in dopaminergic, adrenergic, serotonergic, or cholinergic neurons after this treatment. Rats receiving fetal locus coeruleus tissue implants showed significant post-lesion recovery suggesting that this model can be used to investigate compensatory reinnervation and functional recovery in the central nervous system.

Pain is a salient "stressor" that is mediated by corticotropin-releasing factor-1 receptors

Hummel M, Cummons T, Lu P, Mark L, Harrison JE, Kennedy JD, Whiteside GT
Neuropharmacology Epub, 2010.

Given that corticotrophin-releasing factor (CRF) plays a major role in the response to

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

stress, the authors investigated the role CRF-1 receptors may play in the perception of pain. Both rats and mice received 10 µl-intrathecal injections of 10-µM CRF-SAP (Cat. #IT-13) following a spinal nerve ligation. Administration of CRF-SAP attenuated tactile hypersensitivity, indicating that CRF-1 receptors are involved in pain perception.

Endogenous Histamine Facilitates Long-Term Potentiation in the Hippocampus during Walking

Luo T, Leung LS
J Neurosci 30(23):7845-7852, 2010.

The neurotransmitter histamine is involved in several physiological functions, such as sleep-wake activites, circadian rhythms, learning, and memory. This work examines the role of histamine in modulating synaptic plasticity. Rats received 62.5 ng injections of orexin-SAP (discontinued) into the tuberomammillary nucleus (TMN), followed by assessment of long term potentiation (LTP) during different behavioral states. The data indicate that histaminergic neurons in the TMN facilitate basal-dendritic LTP during walking.

Estrogen therapy and cognition: a review of the cholinergic hypothesis

Gibbs RB
Endocr Rev 31(2):224-253, 2010.

This review discusses estrogen therapy for use in postmenopausal women. In this context the issues revolve around benefits vs. harm of such therapy on the brain and cognitive impairment associated with aging and Alzheimer's disease. Use of 192-IgG-SAP (Cat. #IT-01) to investigate this paradigm is described.

Gene expression profile in rat hippocampus with and without memory deficit

Paban V, Farioli F, Romier B, Chambon C, Alescio-Lautier B
Neurobiol Learn Mem 94(1):42-56, 2010.

This work examined a wide range of gene expression in the rat hippocampus after bilateral injections of 192-IgG-SAP (Cat. #IT-01) – 37.5 ng per side in medial septum and 75 ng per side in nucleus basalis magnocellularis. Memory loss following 192-IgG-SAP treatment was marked by gene expression that did not show the same cluster organization as learning processes. Genes showing differential expression were down-regulated, and one cluster associated with tissue remodeling could be identified.

Role of neurokinin-1 expressing neurons in the locus coeruleus on ventilatory and cardiovascular responses to hypercapnia

de Carvalho D, Bicego KC, de Castro OW, da Silva GS, Garcia-Cairasco N, Gargaglioni LH
Respir Physiol Neurobiol 172(1-2):24-31, 2010.

NK-1 receptors (NK1R) play an important role in cardiorespiratory responses to hypercapnia. In order to paint a clearer picture of the systems involved the authors injected 0.4 µl of 2-µM SP-SAP (Cat. #IT-07) into the locus coeruleus (LC) of rats. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The data suggest that several subpopulations of neurons express NK1R in the LC, and that these subpopulations play different roles in the modulation of cardiorespiratory reponses to hypercapnia.

Splanchnic sympathectomy prevents translocation and spreading of E coli but not S aureus in liver cirrhosis

Worlicek M, Knebel K, Linde HJ, Moleda L, Scholmerich J, Straub RH, Wiest R
Gut 2010.

Advanced cirrhosis activates the sympathetic nervous system. This work investigates the role of the sympathetic nervous system (SNS) in spontaneous bacterial peritonitis – which is mainly caused by translocation of enteric Gram-negative bacteria. Rats received 15-µg intraperitoneal injections of anti-DBH-SAP (Cat. #IT-03). Lesioned animals displayed increased susceptibility to bacterial translocation and infection with *E. coli* but not *S. aureus*. This suggests the SNS plays an important role in the immune response to Gram-negative bacteria.

Noradrenergic neurons of the area postrema mediate amylin's hypophagic action

Potes CS, Turek VF, Cole RL, Vu C, Roland BL, Roth JD, Riediger T, Lutz TA
Am J Physiol Regul Integr Comp Physiol Epub, 2010.

Amylin decreases food intake in rats and is a satiation signal affecting the area postrema (AP). This work investigated the role of noradrenergic neurons in amylin activity. Rats received a total of 50 ng of anti-DBH-SAP (Cat. #IT-03) into the AP and 25 ng into the lateral parabrachial nucleus. Mouse IgG-SAP (Cat. #IT-18) was used as a control. Rats showing a >50% lesion of noradrenergic neurons were unresponsive to low doses of amylin, suggesting that noradrenergic neurons are part of the amylin pathway.

Targeted Ablation of Mesenteric Projecting Sympathetic Neurons Reduces the Hemodynamic Response to Pain in Conscious Spinal Cord Transected Rats

Lujan HL, Palani G, Peduzzi J, Dicarlo SE
Am J Physiol Regul Integr Comp Physiol 298(5):R1358-1365, 2010.

Autonomic dysreflexia is a life-threatening hypertension as a result of a spinal cord injury above thoracic level 6. The authors investigated whether reduction of sympathetic activity can reduce the severity of this condition. Rats received 13.5-µg injections of CTB-SAP (Cat. #IT-14) into the celiac ganglion resulting in ablation of mesenteric-projecting sympathetic neurons. Lesioned animals displayed a reduced pressor response to pain after spinal cord transection, to some extent ameliorating autonomic dysreflexia.

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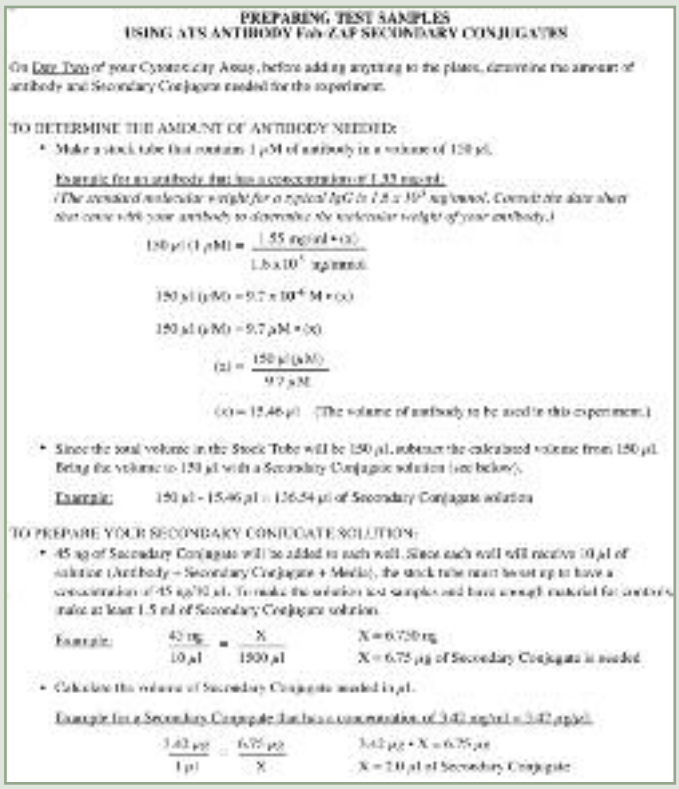
Targeting Talk: Product Questions

by Dr. Douglas Lappi

Fab-ZAP Protocol



PREPARING TEST SAMPLES
USING ATS ANTIBODY Fab-ZAP SECONDARY CONJUGATES

On Day Two of your Cytotoxicity Assay, before adding anything to the plates, determine the amount of antibody and Secondary Conjugate needed for the experiment.

TO DETERMINE THE AMOUNT OF ANTIBODY NEEDED:

- Make a stock tube that contains 1 µM of antibody in a volume of 150 µl.

Example for an antibody that has a concentration of 1.55 mg/ml:
(The estimated molecular weight for a typical IgG is 1.5 x 10^5 mg/mmol. Correct the data sheet that came with your antibody to determine the molecular weight of your antibody.)

$$150\ \mu l\ (1\ \mu M) = \frac{1.55\ mg/ml \cdot (x)}{1.5 \times 10^5\ mg/mmol}$$

$$150\ \mu l\ (\mu M) = 9.7 \times 10^{-6}\ M \cdot (x)$$

$$150\ \mu l\ (\mu M) = 9.7\ \mu M \cdot (x)$$

$$(x) = \frac{150\ \mu l\ (\mu M)}{9.7\ \mu M}$$

$(x) = 15.46\ \mu l$ (The volume of antibody to be used in this experiment.)

- Since the total volume in the Stock Tube will be 150 µl, subtract the calculated volume from 150 µl. Bring the volume to 150 µl with a Secondary Conjugate solution (see below).

Example: 150 µl - 15.46 µl = 134.54 µl of Secondary Conjugate solution

TO PREPARE YOUR SECONDARY CONJUGATE SOLUTION:

- 45 ng of Secondary Conjugate will be added to each well. Since each well will receive 10 µl of solution (Antibody + Secondary Conjugate + Media), the stock tube must be set up to have a concentration of 45 ng/10 µl. To make the solution test samples and have enough material for control's make at least 1.5 ml of Secondary Conjugate solution.

Example: $\frac{45\ ng}{10\ \mu l} = \frac{X}{1500\ \mu l}$ X = 6.750 ng
X = 6.75 µg of Secondary Conjugate is needed

- Calculate the volume of Secondary Conjugate needed in µl.

Example for a Secondary Conjugate that has a concentration of 3.42 mg/ml = 3.42 µg/µl:

$\frac{3.42\ \mu g}{1\ \mu l} = \frac{6.75\ \mu g}{X}$ 3.42 µg • X = 6.75 µg
X = 2.0 µl of Secondary Conjugate

For additional protocols for Fab-ZAP and other ATS products, visit our website:

http://www.atsbio.com/protocols

Fab-ZAP Toxicity

Q: Has anyone from ATS run a titration of Fab-ZAP by itself with Daudi cells (or any cell line) to see if there was any cytotoxicity?

A: While we have not done a titration of the Fab-ZAP line of products, we have tested their cytotoxicity at the maximum concentration used in our in-house assays (45 ng/100 µl, 96-well plate), and have not seen any non-specific killing.

However, with Daudi cells in flow cytometry, we do have some data indicating that the Fab-ZAP (human) binds the cells non-specifically. This appears to be a result peculiar to Daudi cells for undetermined reasons. Fab-ZAP (human) on non-human cell lines has not resulted in non-specific cytotoxicity.

In general this line of products is used as a diagnostic tool with antibodies that are of a particular host species targeted to an alternate species, so the non-specific binding possibility is negligible. Additionally, there are cell lines that are particularly sensitive to Saporin, and Daudi cells may fall into that category, such that it would be a very good idea to titrate the Fab-ZAP product for your own individual application and use.

In Memoriam

By Dr. Douglas Lappi

We commemorate Dr. Dana J. Giulian, MD, PhD, Professor of Neurology at Baylor College of Medicine, who passed away May 9. Dr. Giulian was a widely-published and widely-awarded researcher with an interest in neurotoxins released by microglia. In his efforts to obtain pure hippocampal neurons in primary culture without contaminating microglia, he developed the use of acetylated LDL-saporin to remove microglia (1, 2). Since microglia express scavenger receptors, acetylated LDL was a suitable targeting agent. Fig. 1 illustrates that the method works.

1. Giulian D, Yu J, Li X, Tom D, Li J, Wendt E, Lin S-N, Schwarcz R, Noonan C (1996) Study of receptor-mediated neurotoxins released by HIV-1-infected, mononuclear phagocytes found in human brain. *J Neurosci* 16:3139-3153.

2. Giulian D, Haverkamp LJ, Yu JH, Karshin W, Tom D, Li J, Kirkpatrick J, Kuo YM, Roher AE (1996) Specific domains of b-amyloid from Alzheimer plaque elicit neuron killing in human microglia. *J Neurosci* 16:6021-6037.

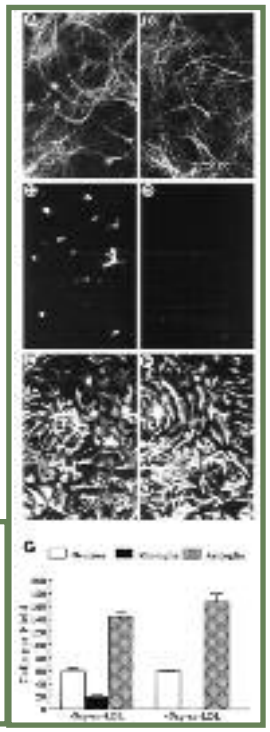


Fig. 1. Selective elimination of microglia from mixed hippocampal cultures. A, C, E, Control cultures show complex neuronal networks revealed by MAP-2/NF immunostaining (A), the presence of DiI-ac-LDL(+) microglia (C), and near-confluent feeder layer of GFAP(+) astrocytes (E). B, D, F, After treatment of cultures with saporin coupled to acetylated LDL, microglia were eliminated (D) without effect on survival of either neurons (B) or astroglia (F). Scale bar, 25 µm. G, Counts of specific cell populations with and without Sap-ac-LDL treatment confirm the specific depletion of microglia. Data are expressed as mean values ± SE obtained from nine randomly selected fields of at least five independent cultures viewed at 200× magnification (2).

Role of Cell Fate Determinants in a Model of Spinal Cord Neurotoxic Lesion Induced by Cholera Toxin B-Saporin

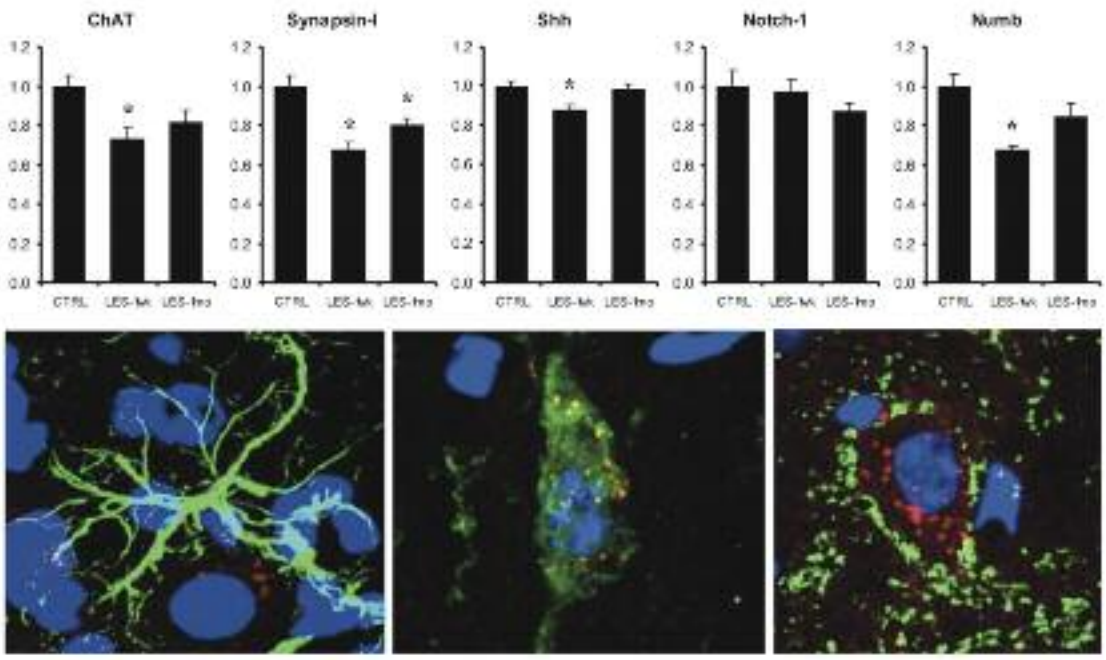


Fig. 2. The graphs show the western blot data relative to the expression levels of ChAT, Synapsin-I, Shh, Notch-1 and Numb in SC homogenates from CTB-SAP lesioned (LES) and control (CTRL) mice. Values are expressed as arbitrary units and normalized to control levels. Asterisks (*) indicate significant difference from control levels. The confocal images show the double-staining with Shh and either GFAP (left), ChAT (middle) or Synapsin-I (right). It is evident that neurons expressed Shh, whereas GFAP-positive astrocytes did not. The same also applies to Notch-1 and Numb staining (not shown).

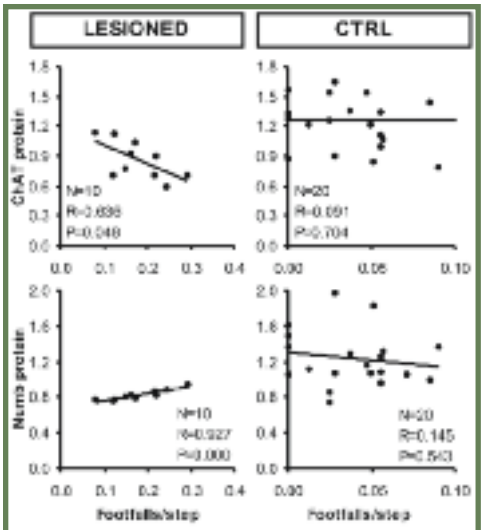


Fig. 3. Linear regression and correlation between motor performance at the grid walk test and protein expression levels. Grid walk performance, expressed as number of footfalls per step, significantly correlated with the expression levels of ChAT and Numb in the lesioned (LES-1wk group), but not in the control (CTRL) group.

(continued from page 1)

influenced by events of synaptic plasticity and that Shh and Notch-1 could be involved in these mechanisms, whereas Numb probably modulates functional recovery by a different mechanism. Given the mentioned roles of Shh, Notch-1 and Numb, we believe that an *in vivo* manipulation of their signalling after lesion could represent a suitable way to improve functional recovery by modulating synaptic plasticity and/or neurogenesis. We believe that our model of SC motoneuron degeneration, induced by CTB-SAP, represents a useful tool for future studies attempting to investigate neurogenesis and/or other compensatory changes within the SC, in the presence of only neurodegenerative processes, without other microenvironmental cues such as inflammation, tissue damage, disruption of SC white matter and blood circulation.

References

1) Edgerton VR, Tillakaratne NJ, Bigbee AJ, de Leon RD, Roy RR (2004) Plasticity of the spinal neural circuitry after injury. *Annu Rev Neurosci*, 27:145-167.

2) Gulino R, Dimartino M, Casabona A, Lombardo SA, Perciavalle V (2007) Synaptic plasticity modulates the spontaneous recovery of locomotion after spinal cord hemisection. *Neurosci Res* 57:148-156.

3) Chen J, Leong SY, Schachner M (2005) Differential expression of cell fate determinants in neurons and glial cells of adult mouse spinal cord after compression injury. *Eur J Neurosci* 22:1895-1906.

4) Costa RM, Honjo T, Silva AJ (2003) Learning and memory deficits in Notch mutant mice. *Curr Biol* 13:1348-1354.

5) Presente A, Boyles RS, Serway CN, de Belle JS, Andres, AJ (2004) Notch is required for long-term memory in Drosophila. *Proc Natl Acad Sci USA* 101:1764-1768.

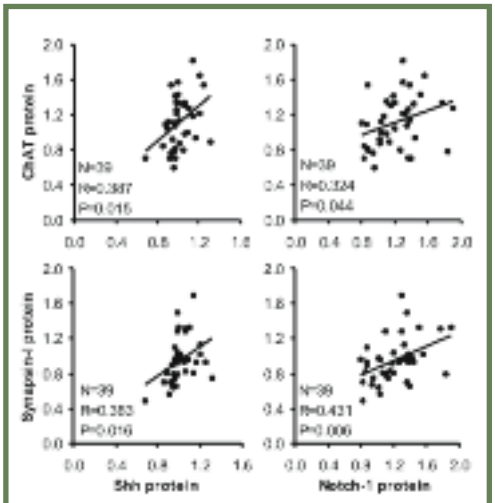


Fig. 4. Linear regression and correlation between protein expression levels in the entire mice population. ChAT expression levels correlated with those of Shh and Notch-1, but not Numb (not shown). Synapsin-I expression levels also correlated with those of Shh and Notch-1, but not Numb (not shown).

SfN 2010 Poster of the Year Award

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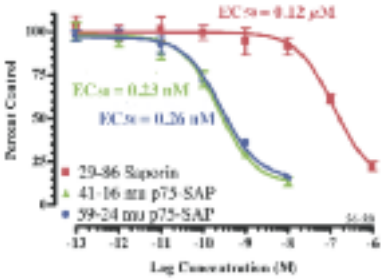
See you in San Diego!

Targeting Tools: Featured Products

Targeting NGF Receptor-Positive Neurons

Targeting in mouse: mu p75-SAP (Cat. #IT-16)

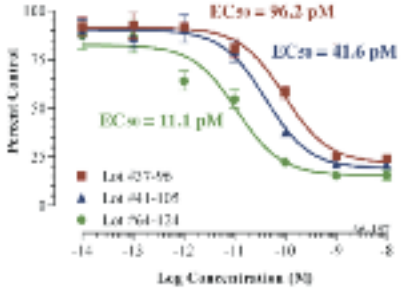
To create this targeted toxin, we affinity-purified the rabbit polyclonal with the immunogen bound to a solid support, and conjugated the affinity-purified antibody (Cat. #AB-N01AP) to saporin. As can be seen in the cytotoxicity assay below, mu p75-SAP has an EC50 in the picomolar range. This greater potency translates to smaller amounts used for elimination of p75-positive neurons in the mouse brain, and results in a greater index of efficacy and lesser non-specific cytotoxicity.



NG3 cells are plated at 1000 cells/well and incubated overnight. Saporin and mu p75-SAP are added in 10-µl volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 1-2 hours, then read at 490 nm.

Targeting in rat: 192-IgG-SAP (Cat. #IT-01)

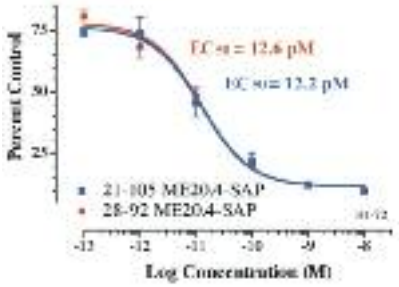
Intraventricular injection of 192-IgG-SAP (192-Saporin) results in almost complete elimination of LNGFR ($p75^{NTR}$)-positive cells in rat. 192-IgG-SAP is directed to a cell-surface antigen that is only expressed at high levels on neurons in the cholinergic basal forebrain (CBF). The antigen, $p75^{NTR}$, is not expressed on the neighboring, non-cholinergic neurons. Visit our website to browse through the more than 370 scientific publications with this powerful targeting tool.



7H6 cells are plated at 1000 cells/well and incubated overnight. 192 IgG-SAP lots are added in 10-µl volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 0.5 to 1 hour, then read at 490 nm.

Targeting in other species: ME20.4-SAP (Cat. #IT-15)

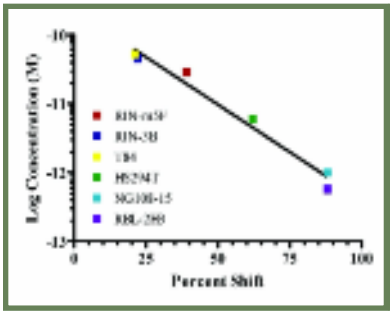
This immunotoxin provides researchers with a powerful lesioning tool — more specific and effective than chemical, surgical or electrolytic lesioning and is active in several species (rabbit, sheep, dog, cat, raccoon, pig and several primate species). Intraventricular injection of ME20.4-SAP has been used to eliminate low-affinity nerve growth factor receptor ($p75^{NTR}$)-positive cells. Tissue-directed injection has also been used in primates to cause loss of $p75^{NTR}$-positive neurons.



HS294T cells are plated at 1000 cells/well and incubated overnight. ME20.4-SAP lots are added in 10-µl volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 1-2 hours, then read at 490 nm.

CTB-SAP (Cat. #IT-14)

CTB-SAP is a conjugate between the cell-binding component of cholera toxin (the B chain) and saporin. CTB binds to GM_1 (monosialotetrahexosylganglioside), which is present on the surface of different neurons. It has been suggested to be involved in many problems (besides the most famous in the gut: cholera) of neuronal systems: Parkinson's, motor neuron degeneration, spinal cord injury, and Alzheimer's disease among others (see cover article).



The percent shift determined by flow cytometry staining with CTB-FITC was plotted against the log concentration of the EC50 in moles/L of CTB-SAP for six cell lines. The data show a distinct correlation between the number of CTB binding sites and the EC50 of CTB-SAP.

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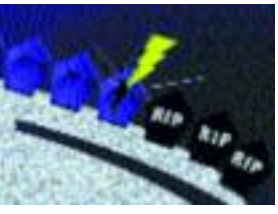

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles taken from the cover story, one letter to each block, to solve the puzzle.

LOREACH

GREENONEUSIS

PUMANAILNOTI

ITTYCLAPIS

CRYLES

What the scientist discovered when he mixed different fields of science.

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

ANSWER: They had . . . _____ _____

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by August 31, 2010.
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See last quarter's winners, page 2.

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Oct-Nov-Dec 2010
Volume 11, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Targeted Ablation of Sympathetic Neurons Reduces Ventricular Arrhythmias and Autonomic Dysreflexia

Contributed by Heidi L. Lujan and Stephen E. DiCarlo,
Department of Physiology, Wayne State University School of Medicine, Detroit, MI 48201

Excessive sympathetic activity is responsible for, and/or contributes to, the morbidity and mortality associated with cardiovascular diseases (e.g. hypertension, stroke, heart failure, ischemic heart disease, ventricular arrhythmias). For example, myocardial ischemia provokes a powerful reflex increase in cardiac sympathetic efferent activity that directly promotes ventricular arrhythmias. Similarly, spinal cord injuries above thoracic level 6 (T6) are associated with episodic bouts of life-threatening hypertension as part of a condition known as autonomic dysreflexia (AD). Physiologically, AD is caused by a massive reflex sympathetic discharge triggered by a stimulus originating below the level of the spinal cord injury.

Importantly, interventions that reduce sympathetic activity protect against ventricular arrhythmias and AD. Accordingly, efforts to reduce sympathetic activity are the first-line therapy for these cardiovascular disorders. However, despite favorable effects, adverse complications (due to generalized sympatho-inhibition, e.g. fatigue, impotence; or specific sympatho-inhibition, e.g. Horner's syndrome, paraesthesia, disruption of sexual, bladder or bowel function) limit compliance and patient satisfaction with these treatments.

However, targeted ablation of cardiac sympathetic neurons reduced the susceptibility to ventricular arrhythmias (2) and targeted ablation of mesenteric projecting sympathetic neurons reduced AD (1) while avoiding these complications.

Specifically, CTB-SAP (cholera toxin B conjugated to saporin; Cat. #IT-14), injected into the stellate ganglia, reduced the number of left ventricular sympathetic fibers (Figure 1), the number of sympathetic post-ganglionic neurons in the stellate ganglia, and virtually eliminated sympathetic pre-ganglionic neurons (SPNs) of spinal cord segments T1-T5 without altering

(continued on page 6)

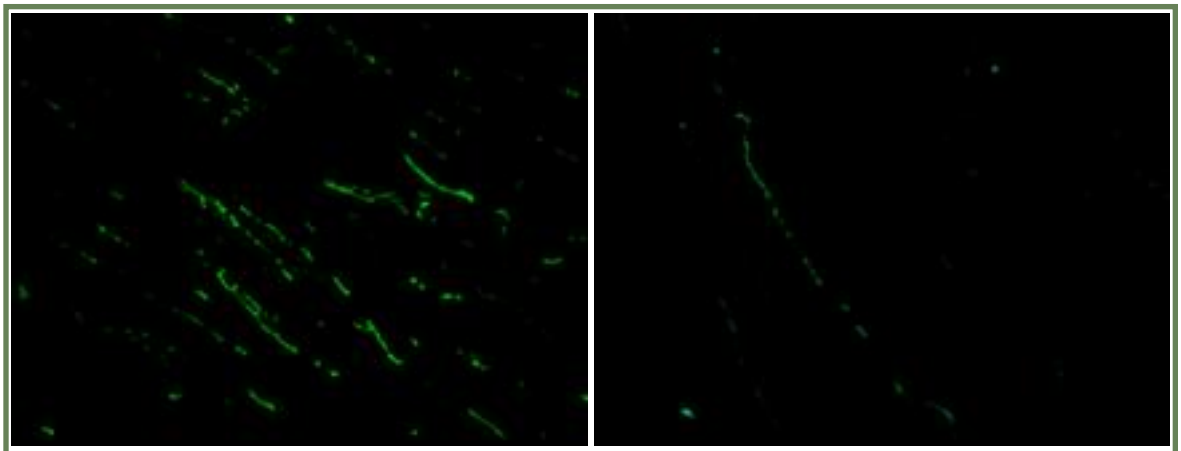

Figure 1 presents tyrosine hydroxylase-immunoreactive sympathetic nerve fibers from the left ventricular free wall of rats that had CTB (left panel) or CTB-SAP (right panel) injected into both stellate ganglia. The CTB-SAP group showed a significant reduction in sympathetic nerve fibers compared to the CTB group.

$3 Million Award to Develop Cancer Pain Drug

Advanced Targeting Systems, the company that pioneered the targeting of specific cell types to manipulate them for the treatment of diseases and for research into the function of biological systems, has been awarded $3 million from the National Cancer Institute (NCI). Representatives from the NCI stated that the Advanced Targeting Systems proposal was ranked #1 for funding out of a nationwide program with hundreds of applicants. Advanced Targeting Systems will use the funds to advance its patented drug, SP-SAP, over the next two years to initiate clinical trials for cancer pain.

SP-SAP is a conjugate between the pain-processing peptide Substance P and the ribosome-inactivating protein saporin. The first publications of SP-SAP in the journal *Science* demonstrated a new direction for the understanding and treatment of pain pathology such as that which accompanies cancer. These have been followed by numerous publications from top-level scientists around the world delineating the activity of SP-SAP. The mechanism of action of SP-SAP is well characterized, a rarity in pain therapeutics: a small number of cells that process pathological pain signals are removed causing relief that appears to be permanent. Normal acute pain is unaffected.

The Food and Drug Administration has recommended that the first population to undergo treatment with SP-SAP is terminal cancer patients who are resistant to opioids such as morphine. Pain due to cancer is a great fear, at times greater than even the fear of death, in the progression of the disease. To make matters worse, many times this pain is unresponsive to the last stand treatment: opioids. There is also a common fear among terminal patients that current pain treatments will leave them unable to function normally at a time when it is personally extremely important for them. In preclinical tests, a single treatment with SP-SAP alleviated pathologic pain perception without affecting other sensory signal pathways.

Advanced Targeting Systems has assembled a team of experts to carry out the goals of the BRDG-SPAN project. Foremost among these are: Dr. Art Frankel from the Scott & White Cancer Research Center, Dr. Allen Burton from the MD Anderson Cancer Center, and Dr. Dorothy Brown of the University of Pennsylvania. All regulatory aspects of the project will be spear-headed by the professional team at Cato Research.

"I am gratified that the National Cancer Institute and the peer reviewers recognize the strength and quality of a 'Dream Team' of physicians, researchers and regulatory specialists that we have put together for this project," stated Dr. Douglas Lappi, Principal Investigator and President/Chief Scientific Officer of Advanced Targeting Systems.

About The BRDG-SPAN Program

The National Institutes of Health BRDG-SPAN Pilot Program (the Biomedical Research, Development, and Growth to Spur the Acceleration of New Technologies Pilot Program (RC3) is supported by funds provided to the NIH under the American Recovery & Reinvestment Act of 2009, a component of the Federal Stimulus Package. The purpose of this pilot program is to accelerate the transition of research innovations and technologies toward the development of products or services that will improve *(continued on page 6)*

Upcoming Events

American Association for Cancer Research
April 2-6, 2011
Orlando, FL

Society for Neuroscience
Nov 14-17, 2010
Booth #3525
San Diego, CA

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: CHOLERA
NEUROGENESIS
MANIPULATION
PLASTICITY
CRESYL

Answer: They had . . . GREAT CHEMISTRY

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

WINNERS: Barbara Attardi, Bioqual, Inc., Rockville, MD * Thea Marlinga, Libertyville, IL * Seto Chice, SUNY-HSC, Brooklyn, NY * Glenn Kageyama, Cal State Poly Univ, Pomona, CA * Harold Schultz, Univ Nebraska Med Ctr, Omaha, NE * April Price, UCSF, San Francisco, CA * Minh Ha, BI Deaconess Med Ctr, Boston, MA * Andrea Chan, Univ California San Diego, La Jolla, CA * Ruth Stornetta, Univ Virginia, Charlottesville, VA

Solve the Teaser online at: www.ATSbio.com/news/10q4_teaser.html

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Mu and delta opioid receptors on nociceptors attenuate mechanical hyperalgesia in rat

Joseph EK, Levine JD
Neuroscience Epub, 2010.

In this work the authors analyzed nociceptor populations mediating mechanical hyperalgesia in the rat. Rats received 3.2 µg of IB4-SAP (Cat. #IT-10) into the subarachnoid space between the L4 and L5 vertebrae. Hyperalgesia due to the administration of NGF was inhibited by DAMGO and SNC even in lesioned animals. These data indicate that most nociceptor populations are involved in mechanical hyperalgesia, and that the mu opioid and delta opioid receptors are co-expressed on some trkA-positive nociceptors.

Induction of CD4(+)CD25(+) T regulatory cells with CD103 depletion

Zikri NN, Schumer E, Wang JJ, Gaughan A, Hadley GA, Moffatt-Bruce SD
J Surg Res 163(1):162-168, 2010.

CD8+ T cells expressing CD103 have been shown to play a key role in the rejection of renal allografts. Use of M290-SAP (a custom saporin conjugation) allows allograft tolerance even in a completely mismatched islet cell transplant model. Use of 1 mg M290-SAP/kg body weight in mice allowed the authors to characterize the kinetics of M290-SAP and its induction of CD4 CD25 regulatory T cells.

Saporin toxin-conjugated monoclonal antibody targeting prostate-specific membrane antigen has potent anticancer activity

Kuroda K, Liu H, Kim S, Guo M, Navarro V, Bander NH
Prostate 70(12):1286-1294, 2010.

Current treatments for prostate cancer are only moderately effective. In this work the authors examined the cytotoxic efficacy of a prostate-specific membrane antigen (PMSA) antibody conjugated to saporin on PMSA-positive cell lines. hJ591, a humanized PMSA antibody, was biotinylated and combined with streptavidin-ZAP (Cat. #IT-27). The hJ591-streptavidin-ZAP complex was specifically cytotoxic to PMSA-positive cell lines, and had anti-cancer activity in a xenograft model. This work demonstrates the anti-cancer potential of targeting PMSA.

Septohippocampal pathways contribute to system consolidation of a spatial memory: Sequential implication of gabaergic and cholinergic neurons

Lecourtier L, de Vasconcelos AP, Leroux E, Cosquer B, Geiger K, Lithfous S, Cassel JC
Hippocampus Epub, 2010.

Few studies have examined the role of GABAergic septohippocampal projections in memory consolidation. The authors administered 192-IgG-SAP (400 ng; Cat. #IT-01) and/or orexin-SAP (70 ng; discontinued) to the medial septum/vertical limb of the diagonal band of Broca of rats. Spatial memory tests were then administered over several weeks. The data indicate that both GABAergic and cholinergic septohippocampal systems contribute to memory stabilization, possibly in a sequential manner.

A new oxytocin-saporin cytotoxin for lesioning oxytocin-receptive neurons in the rat hindbrain

Baskin DG, Kim F, Gelling RW, Russell BJ, Schwartz MW, Morton GJ, Simhan HN, Moralejo DH, Blevins JE
Endocrinology 151(9):4207-4213, 2010.

Evidence suggests that release of oxytocin in the nucleus tractus solitarius (NTS) of the hindbrain can inhibit food intake by augmenting the cholecystokinin satiety response. The authors used oxytocin-SAP (Cat. #IT-46) to eliminate oxytocin receptive cells in the NTS. Blank-SAP (Cat. #IT-21) was used as a control. 0.5 µl-injections of oxytocin-SAP into the NTS caused reduced satiation effect of CCK-8 and blocked the stimulation of food intake by an oxytocin receptor antagonist.

Contribution of limbic norepinephrine to cannabinoid-induced aversion

Carvalho AF, Reyes AR, Sterling RC, Unterwald E, Van Bockstaele EJ
Psychopharmacology (Berl) 211(4):479-491, 2010.

The authors used bilateral injections of anti-DBH-SAP (Cat. #IT-03) into the nucleus accumbens and the bed nucleus of the stria terminalis to investigate the role of neuroepinephrine in cannabinoid-induced aversion and anxiety. Lesioned animals received bilateral 52.5-ng injections of anti-DBH-SAP into the nucleus accumbens or 63 ng into the bed nucleus of the stria terminalis. Saporin (Cat. #PR-01) was used as a control. Lesioned animals displayed reversed aversive behavior, but no change in anxiety-like behavior.

Noradrenergic Nuclei that Receive Sensory Input During Mating and Project to the Ventromedial Hypothalamus Play a Role in Mating-Induced Pseudopregnancy in the Female Rat

Northrop LE, Polston EK, Erskine MS
J Neuroendocrinol 22(10):1061-1071, 2010.

Maintenance of pregnancy or pseudo-pregnancy in rats is maintained by bicircadian prolactin surges induced by vaginal-cervical stimulation. In order to test the hypothesis that medullary noradrenergic cell groups are involved in this process the authors infused rats with either 2 ng or 60 ng anti-DBH-SAP (Cat. #IT-03) into the ventrolateral division of the ventromedial hypothalamus and the posterodorsal medial amygdala. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The data confirm that noradrenergic neurons are involved in the maintenance of pregnancy or pseudopregnancy.

Decrease in membrane phospholipid unsaturation induces unfolded protein response

Ariyama H, Kono N, Matsuda S, Inoue T, Arai H
J Biol Chem 285(29):22027-22035, 2010.

Properties of the cell membrane can be influenced by the degree of fatty acid unsaturation in membrane phospholipids. Alteration of this unsaturation has been

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

implicated in many disease states. Using an SCD1 antibody (Cat. #AB-259) to visualize SCD1 levels by western blot, the authors determined that there are several genetic factors that affect the level of saturated fatty acid in systems modulating insulin resistance, type 2 diabetes, and cardiovascular disease.

NK-1-receptor-mediated lesion of spinal post-synaptic dorsal column neurons might improve intractable visceral pain of cancer origin

Wang Y, Mu X, Liu Y, Zhang X, Wu A, Yue Y
Med Hypotheses Epub, 2010.

There is evidence that spinal post-synaptic dorsal column neurons begin to express neurokinin-1 receptors after visceral stimulation. The authors discuss using this expression profile to target SP-SAP (Cat. #IT-11) to these neurons and eliminate them. This use of 'molecular neurosurgery' may be a replacement for traditional neurosurgery for the treatment of cancer-related visceral pain.

Photochemical internalization (PCI): a technology for drug delivery

Berg K, Weyergang A, Prasmickaite L, Bonsted A, Hogset A, Strand MT, Wagner E, Selbo PK
Methods Mol Biol 635:133-145, 2010.

This review discusses photochemical internalization (PCI), which is a method used to overcome some of the intracellular barriers to introducing molecules into cancer cells. Some difficulties for such therapies include a low rate of release from endocytic vesicles and degradation of the therapeutic molecule by lysosomal enzymes. The use of streptavidin-ZAP (Cat. #IT-27) with a biotinylated EGF receptor antibody is discussed.

Distinct neural pathways mediate alpha7 nicotinic acetylcholine receptor-dependent activation of the forebrain

Thomsen MS, Hay-Schmidt A, Hansen HH, Mikkelsen JD
Cereb Cortex 20(9):2092-2102, 2010.

In this work the authors examine the systems controlling cognitive function in the medial prefrontal cortex (mPFC) and nucleus accumbens shell (ACCshell). Rats received 30-ng injections of 192-IgG-SAP (Cat. #IT-01) into the horizontal limb of the diagonal band of Broca, eliminating the cortically-projecting cholinergic neurons. Deficits in the basal forebrain and the mPFC are shown to be involved in attentional function, while deficits in the ACCshell are shown to be involved in the beneficial effects of antipsychotics on schizophrenia.

Effect of applying p75NTR saporin to a punctured intervertebral disc on calcitonin gene-related peptide expression in rat dorsal root ganglion neurons

Sugiura A, Ohtori S, Yamashita M, Yamauchi K, Inoue G, Suzuki M, Norimoto M, Orita S, Eguchi Y, Kuniyoshi K, Ochiai N, Kishida S, Takaso M, Aoki Y, Ishikawa T, Arai G, Miyagi M, Kamoda H, Nakamura J, Takahashi K
J Orthop Sci 15(3):407-413, 2010.

Lumbar intervertebral discs are suspected to be a source of low back pain, in part because of the innervation of these discs by neurons containing substance P and CGRP receptors. Rats received 2.5 µg of 192-IgG-SAP (Cat. #IT-01) into the L5/6 vertebral disc after the disc was punctured. While half of the dorsal root ganglion neurons innervating the disc were positive for CGRP post-puncture, animals receiving 192-IgG-SAP displayed reduced CGRP expression, indicating a role for the p75 receptor in discogenic pain.

Please visit

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to see a complete list of references using ATS products.

Postnatal development and functional adaptations of the melanopsin photoreceptive system in the albino mouse retina

Gonzalez-Menendez I, Contreras F, Cernuda-Cernuda R, Provencio I, Garcia-Fernandez JM
Invest Ophthalmol Vis Sci 51(9):4840-4847, 2010.

Melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) adjust the circadian pacemaker of mammals by detecting light. The authors tracked the development of ipRGCs in postnatal mice under varying light conditions. Immunohistochemistry for these experiments was done using a melanopsin polyclonal antibody (Cat. #AB-N38). Alteration of the standard light/dark cycle clearly affected the development of ipRGCs.

Noradrenergic neurons of the area postrema mediate amylin's hypophagic action

Potes CS, Turek VF, Cole RL, Vu C, Roland BL, Roth JD, Riediger T, Lutz TA
Am J Physiol Regul Integr Comp Physiol 299(2):R623-631, 2010.

The neuronal pathways used to process the physiological response to amylin were investigated using 50-ng injections of anti-DBH-SAP (Cat. #IT-03) into the area postrema (AP) or 25 ng into the lateral parabrachial nucleus. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The response to amylin administration (reduction of food intake) was significantly reduced in lesioned animals, indicating that noradrenergic neurons in the AP control at least part of this pathway.

Substance P modulation of hypoglossal motoneuron excitability during development: changing balance between conductances

Adachi T, Huxtable AG, Fang X, Funk GD
J Neurophysiol 104(2):854-872, 2010.

This work examined how neuromuscular networks that are immature, but functional, at birth move through development while remaining operational. The authors focused on hypoglossal motoneurons involved in behaviors such as swallowing, suckling, and breathing. Immunohistochemistry was

(continued on page 5)

Targeting Talk: Product Questions

by Dr. Douglas Lappi

Q: In the Targeting Trends *Newsletter, Oct-Nov-Dec 2006 you mentioned mixing anti-DBH-SAP with a tracer, which tracer would you recommend? We were thinking of using FluoroGold. If we do not use a tracer, we were thinking of using a neutral red solution to dilute the stock of anti-DBH-SAP in order to be able to visibly see the toxin being injected into the spinal cord. Could there be an issue of pH if we used neutral red with anti-DBH-SAP? Our concern is that the toxin is not being ejected from the pipette tip or that it is not being taken up into the pipette tip as we can not see it (it's the same color as the mineral oil). We are confident in the targeting of the spinal area for injection as we have previously used FluoroGold only and then were able to visualize it in the area of interest.*

A: Our Scientific Advisor, Dr. Ronald G. Wiley, uses Fast Green dye (0.01-0.1% w/v) in the toxin injection solutions. He originally chose Fast Green because intracellular electrophysiologists had long used it while doing intracellular recordings and shown it was non-toxic. Fast Green has more contrast than Neutral Red (easier to see) and does not affect pH significantly. He has used it with many saporin-containing toxins with success.

Dr. Wiley says, "There are two issues when you talk about using "tracers" with targeted toxins: 1) tracing the acute injection volume to be sure it goes into the animal correctly, and 2) tracing the neurons that projected to the injection site and were therefore susceptible to being killed by the toxin.

Dr. Wiley does not use separate anatomic tracers for the immunotoxins, the only agents taken up and retrogradely transported efficiently. Since ATS immunotoxins are so efficient you have to use a high efficiency tracer such as cholera toxin B (but not WGA since it may not play well with saporin).

Dr. Wiley does not favor FluoroGold (a tin compound) because he has seen some local toxicity at FluoroGold injection sites which might impair uptake and/or transport of a targeted toxin, and it is not clear if it is compatible with saporin-containing toxins.

SfN Meeting Giveaways

Visit the ATS booth (#3525) in San Diego at the 2010 Society for Neuroscience meeting to pick up your tote bag with the famous "3 Guys" Renaissance art. And don't forget your 2011 calendar.

Targeting Topics: Recent Scientific References

(continued from page 4)

performed using an NK-1 receptor antibody (discontinued). The data show that although NK-1 receptor density decreases as the animal matures, substance P (the NK-1 receptor ligand) remains an important part of these networks.

Email ats@ATSbio.com to get answers to your targeting questions.

Orexin-B-saporin lesions in the lateral hypothalamus enhance photic masking of rapid eye movement sleep in the albino rat

Ocampo-Garces A, Ibanez F, Perdomo G, Torrealba F

J Sleep Res Epub, 2010.

Photic masking occurs when photic input to the retina interferes with REM sleep. Rats that received 200 ng of orexin-SAP (discontinued) into the lateral hypothalamus experienced dramatically less REM sleep during normal light cycles. Placing them in a skeleton photoperiod (brief pulses of light, one in the morning and one in the evening), however, caused REM sleep during the rest phase to return to normal. These data suggest that photic masking may explain some effects of narcolepsy and cataplexy.

Targeted Ablation of Sympathetic Neurons Reduces Ventricular Arrhythmias and Autonomic Dysreflexia

(continued from page 1)

afferent neurons. These structural neuroplastic changes were associated with a decreased susceptibility to ischemia-induced sustained ventricular tachycardia.

Furthermore, CTB-SAP injected into the celiac ganglion reduced the number of sympathetic post-ganglionic neurons in the celiac ganglia and virtually eliminated sympathetic pre-ganglionic neurons of spinal cord segments T5-T12 without altering afferent function. Similarly, these neuroplastic changes were associated with a reduced AD.

Thus CTB-SAP retrogradely transported from the peripheral ganglia is effective at ablating specific sympathetic neurons and reducing the susceptibility to ventricular arrhythmias and AD. Additional studies are required to further characterize the physiological responses to this procedure as well as determine if this new approach is safe and efficacious for the treatment of conditions associated with excess sympathetic activity.

References

1. Lujan HL, Palani G, Peduzzi JD and DiCarlo SE. Targeted ablation of mesenteric projecting sympathetic neurons reduces the hemodynamic response to pain in conscious, spinal cord-transected rats. *Am J Physiol Regul Integr Comp Physiol* 298: R1358-R1365, 2010.

2. Lujan HL, Palani G, Zhang L and DiCarlo SE. Targeted Ablation of Cardiac Sympathetic Neurons Reduces the Susceptibility to Ischemia-Induced Sustained Ventricular Tachycardia in Conscious Rats. *Am J Physiol Heart Circ Physiol* 2010.

SfN 2010 Poster of the Year Award

Send your Abstract info to ATS for consideration for this year's award. The winner contributes the cover article in the January 2011 *Targeting Trends* issue, receives $500 product credit and a number of other special ATS gifts. We look forward to seeing your wonderful work.

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Starting a new lab? Waiting for equipment?

Let us test your materials for you. ATS is expert at conducting *in vitro* assays with targeted toxins.
Send us your primary antibody, peptide or protein, ligand, or lectin.
When the *in vitro* results confirm the desired specificity,
ATS can prepare a custom saporin conjugate.

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$3 Million Award to Develop Cancer Pain Drug

(continued from page 2)

human health, help advance the mission of NIH and its Institutes and Centers, and create significant value and economic stimulus. The BRDG-SPAN pilot is intended to help address the funding gap, often called the "Valley of Death", between innovative promising research and development (R&D) and transitioning those innovations to the market, by contributing to the critical funding needed by applicants to carry out later stage research activities and to pursue the next appropriate milestone(s) necessary to move a product/technology along a promising commercialization pathway. This program also aims to foster partnerships among a variety of research and development (R&D) collaborators.

About Advanced Targeting Systems (San Diego, CA)

Advanced Targeting Systems was founded in 1994 as a research reagent company. It has pioneered the use of Molecular Neurosurgery, the use of cell-specific targeting to Activate, Terminate or Stun cells for therapeutic or research purposes. The same principles are now being used by workers studying diabetes, immunology, cancer and other disease states.

About Scott & White Cancer Research Institute (Temple, TX)

The Scott & White Cancer Research Institute (CRI), a non-profit arm of Scott & White Healthcare, is designed to accelerate the development of new therapies for human diseases. Dr. Arthur Frankel heads CRI and is the leading expert on the use of targeted toxins in cancer, having served for more than 20 years in their clinical use.

About M.D. Anderson Cancer Center (Houston, TX)

M.D. Anderson Cancer Center has been selected by US News and World Report, again, as the leading hospital in the United States for cancer treatment. Dr. Allen Burton is Professor and Chair of the Department of Pain Medicine at M.D. Anderson Cancer Center with over 60 publications on cancer chronic pain. He has joined the team to assist in protocol design and to direct the Phase 1/2 clinical study. Dr Burton's department saw over 10,000 patients last year for cancer-related pain.

About Cato Research (HQ: Durham, NC with locations worldwide)

Cato Research is a full-service contract research organization with 20 years of experience. Their highly-qualified team offers integrated drug development services, including CMC, nonclinical, clinical and regulatory strategies as well as clinical trial support for drugs, biologics, diagnostics and medical devices.

Targeting Tools: Featured Products

GAT1-SAP targets GAT-1, the GABA transporter

GAT-1 is a sodium-coupled neurotransmitter transporter responsible for moving γ-aminobutyric acid (GABA) across cell membranes. GABA is the predominant inhibitory neurotransmitter in the mammalian central nervous system. GAT-1 is widely distributed in both the central and peripheral nervous systems. GAT-1 and GABA are present in numerous neuronal pathways, some of which are implicated in epilepsy, sleep disorders, neuropathic pain, and attention deficit disorders.

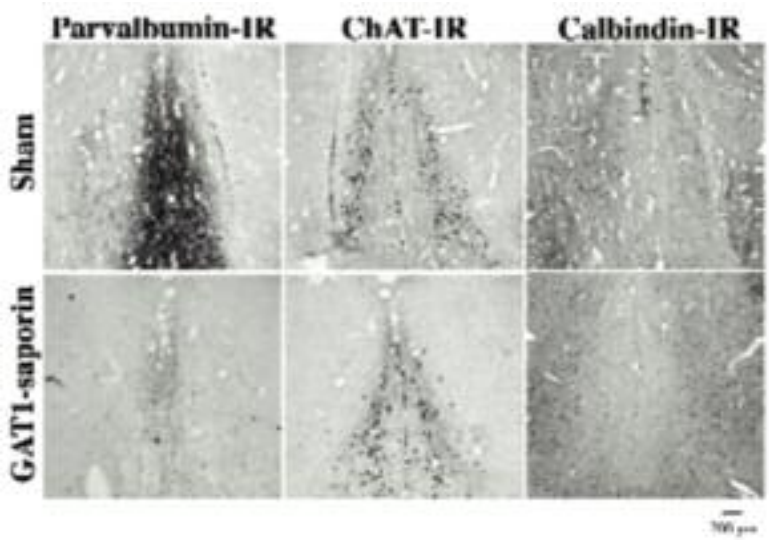
We have constructed a conjugate between an antibody to an extracellular domain of GAT-1 and saporin, the ribosome-inactivating protein. This construct, GAT1-SAP (Cat. #IT-32) has been used to specifically remove GABAergic neurons of the anterior bed nucleus of the stria terminalis (1) and the medial septum and diagonal band of rats (2). Figure 1 shows the specificity of the targeted toxin, with parvalbumin-positive neurons being drastically reduced, while most cholinergic neurons are spared.

Coming soon is our vesicular GABA transporter-saporin construct to increase the ability to eliminate specific populations of GABAergic neurons.

References

1. Radley JJ, Gosselink KL, Sawchenko PE. (2009) A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. *J Neurosci* 29(22):7330-40.

2. Pang KC, Jiao X, Sinha S, Beck KD, Servatius RJ. (2010) Damage of GABAergic neurons in the medial septum impairs spatial working memory and extinction of active avoidance: Effects on proactive interference. *Hippocampus*. Epub, May 17.



Immunocytochemistry following sham surgery (top row) or administration of GAT1-SAP (bottom row, 325 ng/µl) into the medial septum-diagonal band of Broca (MSDB). Staining of parvalbumin-immunoreactive neurons in the MSDB was dramatically reduced following GAT1-SAP administration (left). Parvalbumin-ir neurons in the MSDB are GABAergic septohippocampal neurons. In contrast to parvalbumin-ir neurons, neither cholinergic neurons (ChAT-ir, middle) nor calbindin-ir neurons (right) were altered following GAT1-SAP application in the MSDB. Scale bar - 200 µm. Figure provided by KCH Pang *et al.* (2008) *Targeting Trends* 9(1).

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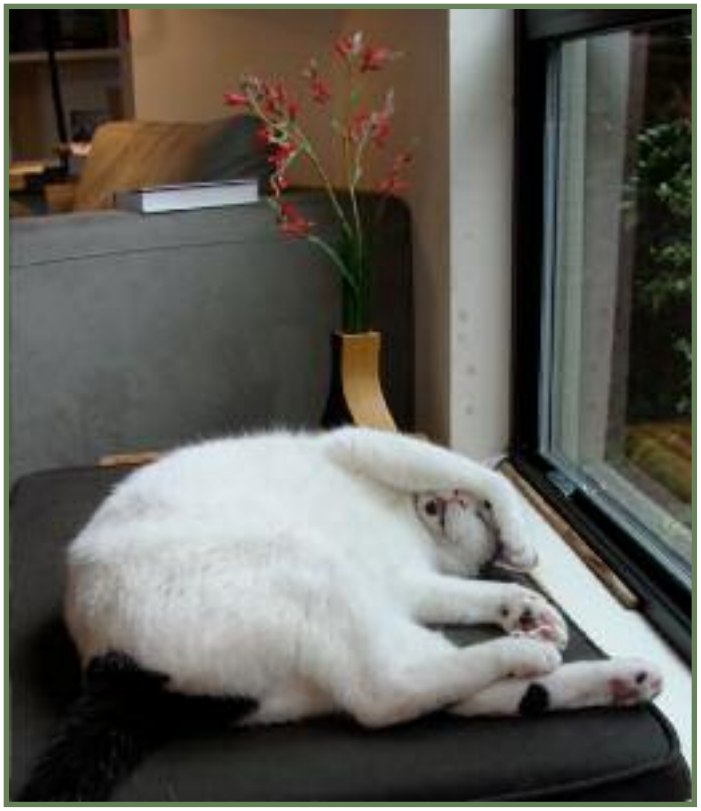
GAT1-SAP, Cat. #IT-32

available in these sizes:
25 micrograms, 100 micrograms, and 250 micrograms

Kit includes the following controls in equal amounts:
Saporin, Cat. #PR-01
GAT-1 Rabbit Polyclonal, Cat. #AB-N37
Rabbit IgG-SAP, Cat. #IT-35



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Choose an ANTIBODY§ specific to your cell type.

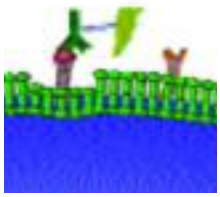
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

§ or anything recognized on the cell surface and internalized.

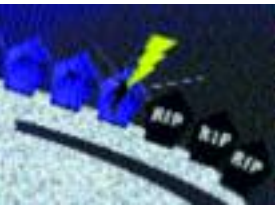
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



Cells that 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 taken from the cover story, one letter to each block, to solve the puzzle.

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DERBALD

TENMEGS

BRIEFS

SPOIDICE

Why the boy thought his scientist costume was the best.

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

ANSWER: He had an . . .

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page or complete online with the correct solution by November 30, 2010.
3. Win $100 credit toward your next purchase.

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