Jan-Feb-Mar 2009
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Targeting Trends

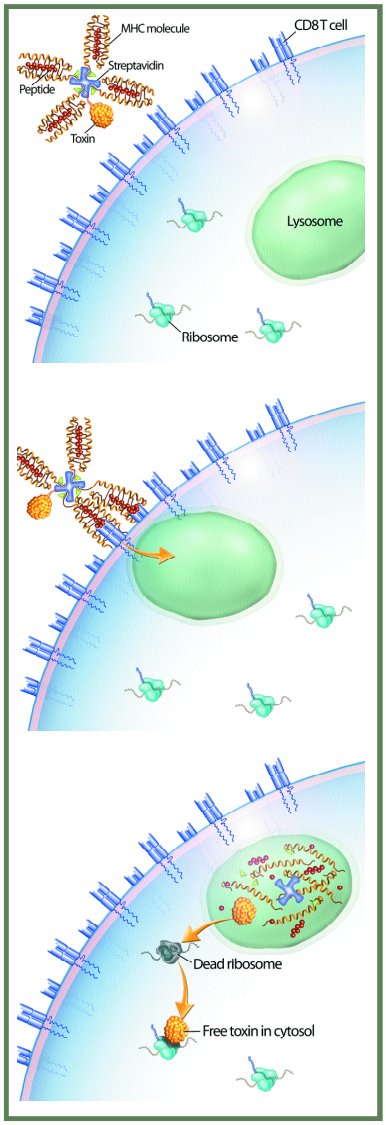
Reporting the latest news in Molecular Surgery

Selective deletion of CD8+ T cells by saporin-coupled MHC class I tetramers

Contributed by Paul R. Hess, Adam S. Buntzman, Sabrina L. Murray, Ellen F. Young, and Jeffrey A. Frelinger; North Carolina State University College of Veterinary Medicine, Raleigh, NC; and University of North Carolina-Chapel Hill School of Medicine, Chapel Hill, NC.

CD8+ T cells constitute important effectors of the adaptive immune response, functioning principally to remove infected cells from the body, which are detected by the display of short peptides (epitopes) derived from microbial proteins within the binding groove of class I major histocompatibility complex (MHC) molecules on the cell surface. When the T cell receptor (TCR) of a primed T cell binds to its cognate peptide-MHC (pMHC) ligand, the T cell is triggered, and induces apoptosis in the infected cell. To anticipate the potential myriad of pathogen-origin peptides that might be encountered over a lifetime, a correspondingly large, diverse TCR repertoire is randomly generated, with each nascent T cell expressing thousands of identical TCRs of a single specificity. During the subsequent selection process that occurs in the thymus, most T cells bearing TCRs that inadvertently bind MHC molecules presenting "self" peptides (i.e., derived from normal proteins) are deleted prior to entering the circulation, to prevent autoimmunity; the minority of these autoreactive T cells that escape elimination are turned off by peripheral tolerizing mechanisms. In some immune-mediated conditions, such as multiple sclerosis and type 1 diabetes mellitus, normal tolerance is disabled, and autoreactive CD8+ T cells are inappropriately activated, leading to organ-specific tissue destruction and clinical signs of disease. Unfortunately, non-specific inhibition of T cell responses with immunosuppressive agents has not been particularly effective for these conditions, and such drugs carry risks of cancer and serious infections. Selective deletion of the pathogenic CD8+ T cells would appear to be an ideal strategy, but, until recently, there has been no efficient means of targeting just the culprits. In 1996, Altman *et al*. showed that CD8+ T cells of known specificity could be discriminated from other T cells in polyclonal populations by the use of soluble complexes, widely known as "tetramers," consisting of four identical pMHC molecules bound to streptavidin.[1] When coupled to a fluorophore, such tetramers permit ready visualization of epitope-specific T cells by flow cytometry.

(continued on page 6)



Artist's conception of an MHC class I toxic tetramer
(illustration: AM Harvey, NCSU-CVM).

Inside this issue:

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Denise Higgins, Editor
Gangsta, Guest Editor

ADVANCED TARGETING SYSTEMS

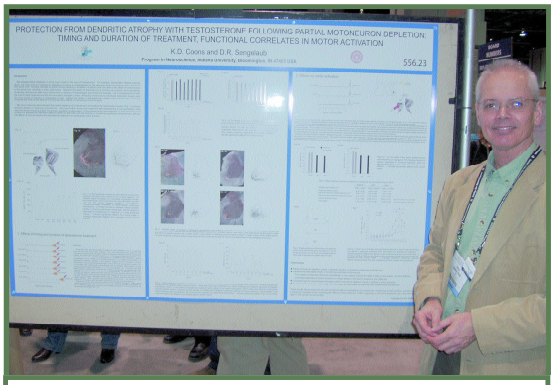
Annual Society for Neuroscience Poster Award

This year's winner of Poster of the Year goes to Arshad Khan of USC for his poster: *Stimulus-, circuit- and intracellular-level determinants of MAP kinase and CREB activation in parvicellular hypothalamic paraventricular neurons*. AM Khan, KL Rapp, TA Ponzio, G Sanchez-Watts, AG Watts. Dr. Khan's work will be featured on the cover of the next issue of *Targeting Trends*.

Dr. Khan's poster continues the researchers' work on catecholaminergic neurons and feeding. The featured experiments involved downstream effects on signal transduction when something is missing, in this case, catecholaminergic neurons. The study followed on their work published in 2007 (*J. Neurosci*. 27:7344-7360) which asked if there were a causal relationship of noradrenergic neurons in the system. Anti-DBH-SAP (Cat. #IT-03) allowed this to be determined.

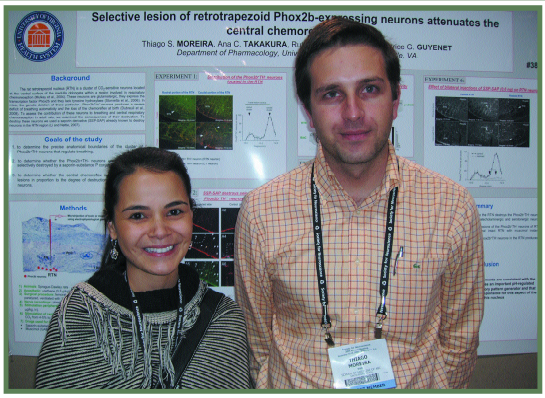
Doug Lappi presents Arshad Khan with this year's ATS Neuroscience Poster Award

There were many great abstracts using ATS products, and this was a very difficult year for deciding the winner. Runners-up (and who knows how these selections are made!) included these fine posters.



2008 SFN Award Runner-up Dale Sengelaub

Dale Sengelaub presented his latest work also looking at the effects of something missing: *Protection from dendritic atrophy with testosterone following partial motoneuron depletion: Timing and duration of treatment, functional correlates in motor activation*. KD Coons, DR Sengelaub. Partial loss, due to CTB-SAP (Cat. #IT-14), results in dendritic atrophy of survivor motorneurons, and this work shows administration of testosterone has a neuroprotective effect. A beautiful poster.



2008 SFN Award Runner-up Thiago Moreira and Ana Takakura

The poster presented by Thiago Moreira and Ana Takakura (equal contributors) continues the work from this Brazilian pair with the Guyenet laboratory. concerning the control of central chemoreflex from the retrotrapezoid nucleus: *Selective lesion of retrotrapezoid Phox2b-expressing neurons attenuates the central chemoreflex in rats*. TS Moreira, AC Takakura, RL Stornetta, PG Guyenet. SSP-SAP (Cat. #IT-11) was used to specifically delete a small number of cells, but reaching a threshold to show a behavioral effect. Ann Schreihofer was a Poster of the Year winner in 1999 when she was in the Guyenet lab.

And last, but not least, Mark Baxter of the University of Oxford, Department of Experimental Psychology, used ME20.4-SAP (Cat. #IT-15) to eliminate cholinergic neurons in the dorsolateral prefrontal cortex, resulting in difficulty of memory maintenance in feeding tasks: *Cholinergic depletion of prefrontal cortex impairs acquisition of the delayed response task in rhesus monkeys*. MG Baxter, DA Kyriazis, PL Croxson. Dr. Baxter's molecular surgery, requiring numerous injections, was a technical *tour de force*.

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Neuropathic pain is maintained by brainstem neurons co-expressing opioid and cholecystokinin receptors

Zhang W, Gardell S, Zhang D, Xie JY, Agnes RS, Badghisi H, Hruby VJ, Rance N, Ossipov MH, Vanderah TW, Porreca F, Lai J
Brain [Epub Dec 2], 2008.

It has been hypothesized that a subset of rostral ventromedial medulla (RVM) neurons co-expressing the cholecystokinin type 2 receptor and the mu-opioid receptor are responsible for the maintenance of neuropathic pain. Rats were treated with 50-ng bilateral RVM injections of Dermorphin-SAP (Cat. #IT-12), CCK-SAP (Cat. #IT-31), or saporin (Cat. #PR-01) as a control. Lesion of the RVM neurons prevented hyperalgesia in response to CCK treatment, and shortened abnormal pain states caused by sciatic nerve injury.

Cardiac Damage after Lesions of the Nucleus Tractus Solitarii

Nayate A, Moore SA, Weiss RM, Taktakishvili O, Lin LH, Talman WT
Am J Physiol Regul Integr Comp Physiol [Epub Nov 19], 2008.

Specific neurokinin-1 (NK-1) receptor-expressing neuron lesions in the nucleus tractus solitarii (NTS) have led to the unexplained death of treated rats. In this work the authors examined cardiac specific parameters in rats after administration of 9.4 ng of SSP-SAP (Cat. #IT-11). The SSP-SAP was directed to either the dorsolateral and medial portions of the NTS, or into the brain stem outside of the NTS as a control. The data suggests that NTS lesion interrupting the baroreflex may induce cardiac arrythmias and other myocardial changes leading to sudden cardiac death.

Endosialin protein expression and therapeutic target potential in human solid tumors: sarcoma versus carcinoma

Rouleau C, Curiel M, Weber W, Smale R, Kurtzberg L, Mascarello J, Berger C, Wallar G, Bagley R, Honma N, Hasegawa K, Ishida I, Kataoka S, Thurberg BL, Mehraein K, Horten B, Miller G, Teicher BA
Clin Cancer Res 14(22):7223-7236, 2008.

Endosialin is an antigen expressed in many human cancer cell lines. As part of a wide-ranging study investigating clinical specimens, cell culture, and animal models, this group used Hum-ZAP (Cat. #IT-22) combined with a humanized anti-endosialin antibody in cell proliferation assays. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The anti-endosialin antibody and Hum-ZAP were incubated together in equimolar concentrations then applied to cells in culture in 0.5 pM to 50 nM concentrations. Various cancers, including synovial sarcoma, fibrosarcoma, and osteosarcoma among others, were found to express endosialin.

Attentional demands for demonstrating deficits following intrabasalis infusions of 192 IgG-saporin

Burk JA, Lowder MW, Altemose KE
Behav Brain Res 195(2):231-238, 2008.

Attentional processing has been shown to be dependent on basal forebrain cholinergic inputs to the cerebral cortex. In this work the authors wished to specify which components should be used to demonstrate deficits following the loss of these neurons. Rats received 200 ng intrabasalis infusions of 192-IgG-SAP (Cat. #IT-01). Testing of lesioned animals indicated that attentional deficits are due to increase of overall attentional task demands as opposed to any single task parameter.

Organization of food protection behavior is differentially influenced by 192 IgG-saporin lesions of either the medial septum or the nucleus basalis magnocellularis

Martin MM, Winter SS, Cheatwood JL, Carter LA, Jones JL, Weathered SL, Wagner SJ, Wallace DG
Brain Res 1241:122-135, 2008.

In this work the authors used a food-protection model to investigate the role of cholinergic neurons in the processing of information from internal and external sources. Rats received the following amounts of 192-IgG-SAP (Cat. #IT-01): 15 ng or 20 ng into the medial septum (MS), or 20 ng into the nucleus basalis magnocellularis (NB). While the NB lesions reduced the number of successful food protection behaviors, lesions in the MS disrupted the temporal organization of this behavior.

(continued on page 4)

Please visit www.ATSbio.com to see a complete list of references.

Targeting Topics: Recent Scientific References

(continued from page 3)

Selective lesion of septal cholinergic neurons in rats impairs acquisition of a delayed matching to position T-maze task by delaying the shift from a response to a place strategy

Fitz NF, Gibbs RB, Johnson DA
Brain Res Bull 77(6):356-360, 2008.

It has been theorized that the effect of cholinergic lesions of the medial septum on learning depend on the stressful nature of the task being learned. The authors injected 0.2 μg of 192-IgG-SAP (Cat. #IT-01) into the medial septum of rats, then examined the strategies used by these animals to learn a delayed matching to position T-maze task. Lesioned animals were less able to switch from one strategy to another, indicating that this mechanism is the primary one affected by septal cholinergic lesions.

Selective lesion of medial septal cholinergic neurons followed by a mini-stroke impairs spatial learning in rats

Craig LA, Hong NS, Kopp J, McDonald RJ
Exp Brain Res [Epub Oct 21], 2008.

Recent work has suggested that reduced levels of acetylcholine, seen in Alzheimer's disease patients, increases the susceptibility of hippocampal neurons to future challenges. Rats received two injections totaling 7.5 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum/vertical limb of the diagonal band of Broca. The vasoconstrictor endothelin-1 was used to create small localized strokes in the hippocampus of lesioned animals. The data suggest that loss of these hippocampal neurons compromises functional recovery from stroke.

Please visit www.ATSbio.com to see a complete list of references.

Descending facilitation from the brainstem determines behavioural and neuronal hypersensitivity following nerve injury and efficacy of pregabalin

Bee LA, Dickenson AH
Pain 140(1):209-223, 2008.

Rostral ventromedial medulla (RVM) facilitatory On cells are thought to be involved in the mechanisms that control chronic pain. Dermorphin-SAP (Cat. #IT-12, 3 pmol) injected into the RVM of rats was used to examine how mu-opioid receptor-expressing facilitatory cells fit into this circuit. Saporin (Cat. #PR-01) was used as a control. The results show that activity in the RVM may influence the outcome of nerve injury.

The role of cholinergic basal forebrain neurons in adenosine-mediated homeostatic control of sleep: lessons from 192 IgG-saporin lesions

Kalinchuk AV, McCarley RW, Stenberg D, Porkka-Heiskanen T, Basheer R
Neuroscience 157(1):238-253, 2008.

The level of adenosine in the basal forebrain increases during sleep deprivation (SD). The cholinergic system of the basal forebrain is thought to be involved in the control of this process. 0.23 μg of 192-IgG-SAP (Cat. #IT-01) was injected into the horizontal diagonal band/ substantia innominata/ magnocellular preoptic nucleus, or 6 μg into the lateral ventricle of rats. The time course was dependent on the injection site, but eventually the SD-induced increase in adenosine was virtually eliminated.

Cholinergic depletion of the medial septum followed by phase shifting does not impair memory or rest-activity rhythms measured under standard light/dark conditions in rats

Craig LA, Hong NS, Kopp J, McDonald RJ
Brain Res Bull [Epub Nov 24], 2008.

It has been theorized that cognitive decline observed in Alzheimer's disease is in part due to disruption of the circadian rhythm (CR) in these patients. Some basal forebrain cholinergic neurons project to the suprachiasmatic nucleus, which is responsible for maintenance of CR. Rats received two injections totaling 7.5 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum/diagonal band of Broca. Lesioned animals did not show any evidence of CR disruption.

Targeted destruction of photosensitive retinal ganglion cells with a saporin conjugate alters the effects of light on mouse circadian rhythms

Goz D, Studholme K, Lappi DA, Rollag MD, Provencio I, Morin LP
PLoS ONE 3(9):e3153, 2008.

Retinal ganglion cells expressing melanopsin photopigment are thought to be involved in non-image forming visual responses to light. The authors had a custom conjugate made between saporin and an anti-melanopsin antibody. A 400-ng injection of the melanopsin-SAP (now available as Cat. #IT-44) conjugate into the eye of a mouse resulted in a loss of the targeted cells. Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The data indicates that melanopsin-containing cells are involved in the response to certain non-image forming visual input.

Targeting Talk: Anti-IgM-ZAP

by Dr. Douglas Lappi

Q: We were wondering how an IgM primary antibody might work in a Mab-ZAP assay. I realize that the conjugated antibody is an anti-IgG whole molecule antibody. However there may well be aspects/epitopes shared in common between IgG and IgM that might render an IgM primary useful with the Mab-ZAP reagent… or not? Has anyone looked at this with your products?

A: We do believe, but have not confirmed, that you will see a cross-reactivity, but at a lower level. We do sell a second immunotoxin for IgM's, Anti-M-ZAP (Cat. #IT-30) which is made from a goat anti-murine IgM.

Anti-M-ZAP

(Cat. #IT-30)

A "second" immunotoxin that relies on your antibody for cytotoxicity to target cells.

Elimination of Specific Cell Type

• Cells that internalize your mouse monoclonal IgM antibody will be eliminated.

*

• Potency may vary according to the specificity and affinity of YOUR antibody to ITS receptor.

*

• Anti-M-ZAP is most effective in determining specificity of your antibody and suitability for conjugation as a primary immunotoxin.

Experimental Biology
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Booth #218

Amer Assoc Cancer Research
April 18-22
Denver, CO
Booth #1760

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: AMOEBA
GRANULAR
PROTEIN
CULTURE
INCUBATOR

Answer: The . . .BIG PICTURE

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

WINNERS: Glenn Kageyama, Cal Poly Pomona Univ, Pomona, CA * Sophie Lopen, Methodist Hospital Res Inst, Houston, TX * Caroline Kent, Mayo Clinic, Jacksonville, FL * Aamir Ahmad, Karmanos Cancer Inst, Detroit, MI * Kumuda Saraff, Cal State Univ, Northridge, CA * April Price, Univ California, San Francisco, CA * Thea Marlinga, Libertyville, IL

Featured Antibodies

NGFr (mu p75) Rabbit Polyclonal

Cat. #AB-N01, 100 μl or affinity-purified Cat. #AB-N01AP, 50 μg

Recognizes $p75^{NTR}$ in mouse. The antisera was developed in rabbit using an extracellular fragment from the mouse p75 receptor (amino acids 43-161). The antibody was affinity-purified using the extracellular domain of p75.

Applications: immunohistochemistry (frozen or paraffin-embedded cells and tissue; 1:150), immunoprecipitation, immunoblotting (1:2,000), flow cytometry (1:1,000), and blocking the function of NGFr (1:1,000).

NGFr (ME20.4, p75) Mouse Monoclonal

Cat. #AB-N07, 100 μg

Recognizes the $p75^{NTR}$ (low affinity neurotrophin receptor) in human, primate, rabbit, sheep, dog, cat, and pig. It was derived from immunization of mice with WM245 melanoma cells.

Applications: flow cytometry (1:100), immunoprecipitation, immunohistochemistry (frozen), electron microscopy (1:200), immunocytochemistry (10 ng/ml), and radioimmunoassay.

Selective deletion of CD8+ T cells by saporin-coupled MHC class I tetramers *(continued from page 1)*

We and others have shown that, after binding to the TCR, tetramers are endocytosed by the T cell.[2, 3] These two characteristics – specific binding and rapid internalization – suggested that tetramers might be a useful way to selectively deliver an intracellularly-active toxin to pathogenic T cells. To investigate this hypothesis, we used TCR-transgenic P14 mice as a source of CD8+ T cells, which recognize a viral glycoprotein-derived peptide, gp33, presented by the class I MHC molecule, H2-Db, and bind to the tetramer, gpC9M. To confirm our observations with a second epitope, we employed TCR-transgenic HY mice, whose CD8+ T cells bind to the H2-Db tetramer, hyC2A. Toxic tetramers were generated from gpC9M and hyC2A pMHC monomers using streptavidin-saporin (SA-SAP; Cat. #IT-27). After assembly, these tetramers retained the TCR-binding specificity of their fluorophore-labeled counterparts, and inhibited translation in a cell-free assay as potently as parent SA-SAP alone.[3] To determine whether T cells would efficiently internalize SAP-coupled tetramers, we briefly cultured quiescent P14 T cells with the gpC9M-SAP tetramer, or as negative controls, with non-toxic gpC9M (not shown) or toxic hyC2A-SAP tetramers. Following the addition of FITC-labeled SAP antibody (Cat. #FL-02), T cells were subsequently incubated at either 37°C or 4°C, which permitted or prohibited endocytosis, respectively. To discriminate internal and external fluorescence, tetramer-antibody-fluorophore complexes on the surface were either removed with an acetic acid solution ("stripped"), or allowed to remain intact ("washed"), prior to analysis. As shown in Fig. 1, acid-resistant fluorescence (gray line), corresponding to endocytosed SAP, was found in all metabolically-active P14 T cells incubated with the cognate gpC9M-SAP, but not with control tetramers. We next evaluated the ability of the SAP-coupled tetramers to kill T cells *in vitro*. Purified P14 and HY T cells were incubated with either non-toxic tetramers alone; non-toxic tetramers plus free (unbound) SAP; or SAP-coupled relevant or irrelevant tetramers. Surviving cells were identified by exclusion of a membrane-impermeant dye, 7-aminoactinomycin-D, at the time points shown in Fig. 2A. Some cell loss was observed over time with all treatments, characteristic of stimulated, cultured T cells; however, incubation of HY T cells with hyC2A-SAP resulted in the death of 98% of cells within a 3-day period. Free SAP was not toxic to T cells. *In vitro* killing of these CD8+ T cells by the cognate SAP-coupled tetramers depended on the tetramer dose (Fig. 2B), and the avidity of the tetramer-TCR interaction (not shown).

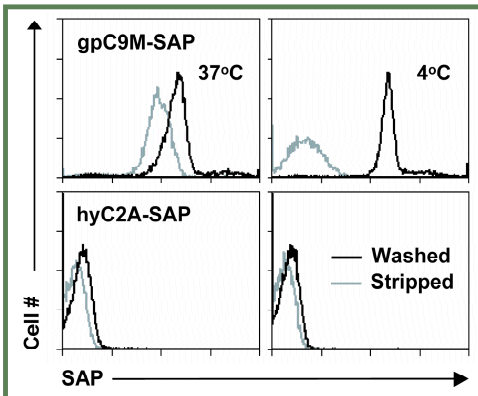


Fig. 1. All metabolically-active (37°C) P14 T cells incubated for 1 hour with the cognate tetramer gpC9M-SAP (5 nM) have endocytosed SAP. Stripped cells show only internal SAP; washed cells show total (internal & external) SAP.

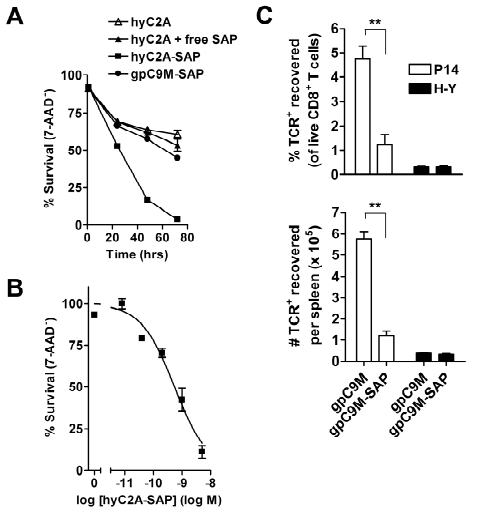


Fig. 2. A. Virtually all HY T cells incubated with 5 nM hyC2A-SAP tetramer are killed by 72 h. Neither free SAP nor SAP coupled to the gpC9M-SAP caused cell death above the background seen with non-toxic tetramer. **B.** HY T cells incubated for 72 h with varying concentrations (5 – 0.008 nM) of the SAP-coupled cognate tetramer are killed in a dose-dependent fashion. **C.** Intravenous injection of gpC9M-SAP tetramer (22 pmol) resulted in significant loss of adoptively-transferred P14 T cells in the spleens of recipient C57BL/6 mice 3 d after administration; recovery of co-transferred control HY T cells did not differ between treatments. The top and bottom panels show results as cell % and cell #, respectively.
**P<0.005 by 2-tailed Student's t test.

We then sought to determine if our toxic tetramers could delete specific CD8+ T cells *in vivo*. Fluorophore-labeled tetramers, when injected intravenously, rapidly bind to cognate T cells in lymph nodes, spleen, and bone marrow, suggesting that SAP-coupled tetramers similarly should be able to reach their targets. To test this hypothesis, we transferred P14 T cells into recipient mice, and after 24 h, administered either gpC9M or gpC9M-SAP. Fig. 2C shows that, after 3 d, >75% of P14 cells were deleted from the spleen in gpC9M-SAP-treated mice; the recovery of control HY cells was not different between treatment groups. At this dosage, injection of the SAP-coupled tetramers caused an acute, mild liver injury, but mice showed no clinical signs of illness.[3]

These studies showed that tetrameric pMHC complexes can be used to deliver a potent toxin, SAP, to epitope-specific CD8+ T cells *in vitro* and *in vivo*, leading to deletion of the target population. Such toxic tetramers could represent a novel and effective means for eradicating pathogenic T cell responses in selected immune-mediated diseases.

References:

1. Altman JD, *et al.* (1996) Phenotypic analysis of antigen-specific T lymphocytes. *Science* 274(5284):94-96.
2. Whelan JA, *et al.* (1999) Specificity of CTL interactions with peptide-MHC class I tetrameric complexes is temperature dependent. *J Immunol* 163(8):4342-4348.
3. Hess PR, *et al.* (2007) Selective deletion of antigen-specific CD8+ T cells by MHC class I tetramers coupled to the type I ribosome-inactivating protein saporin. *Blood* 109(8):3300-3307.

Targeting Technology

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

Choose an ANTIBODY§ specific to your cell type.

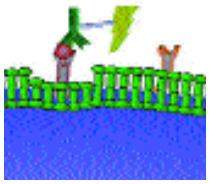
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

§ or anything recognized on the cell surface and internalized.

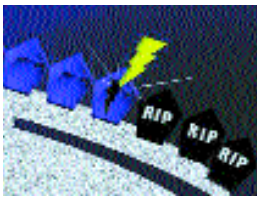
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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, one letter to each block, to solve the puzzle.

TREEMART

ECOTANG

SHAMEMINC

EFTINC

CLEETS

What the scientist looked for in the test tube.

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

ANSWER:

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, 2009
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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Apr-May-Jun 2009
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Targeting Trends

Reporting the latest news in Molecular Surgery

Deletion of Catecholaminergic Neurons by Anti-DBH-Saporin Disrupts Hypothalamic MAP Kinase and CREB Activation

Contributed by Arshad M. Khan, Kimberly L. Rapp, Todd A. Ponzio, Graciela Sanchez-Watts and Alan G. Watts, Dept. of Biological Sciences and Neuroscience Research Institute, University of Southern California, Los Angeles, CA; and Laboratory for Neurochemistry, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD. 2008 SfN Poster Award Winner.

The brain has evolved adaptive mechanisms for coping with stress and responds to stressors in highly stereotyped ways. One of the major physiological responses to stressful stimuli, the secretion of pituitary and adrenal hormones, is controlled by corticotropin-releasing hormone (CRH)-expressing neurons located in the paraventricular nucleus of the hypothalamus (PVH). CRH neuroendocrine neurons constitute the primary control center in the brain for initiating hormonal responses to stress, and the control of these neurons by other parts of the brain has been the subject of intensive investigation. One of the most massive sources of input to these neurons is the collection of axonal inputs originating from subpopulations of catecholaminergic (CA) neurons located in the hindbrain. These CA neurons are critical regulators of the mammalian stress axis,

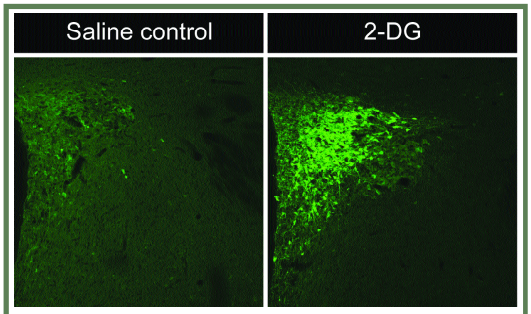

Figure 1. Levels of phospho-ERK1/2 (green signal) are elevated in the PVH in animals receiving intravenous 2-DG, as compared to saline-injected control animals.

releasing the neurotransmitters epinephrine, norepinephrine and other co-localized peptide hormones (such as neuropeptide Y) onto CRH neuroendocrine neurons.

A key question about this input system concerns its role in carrying information to the CRH neuroendocrine system to mediate responses to various stressors. We have previously shown that glycemic challenges such as 2-deoxyglucose (2-DG) or insulin, both of which produce changes in glucose concentrations when injected intravenously, trigger activation of CRH neuroendocrine neurons and are also associated with increases in the plasma concentrations of stress hormones[1,2]. The activation of

(continued on page 6)

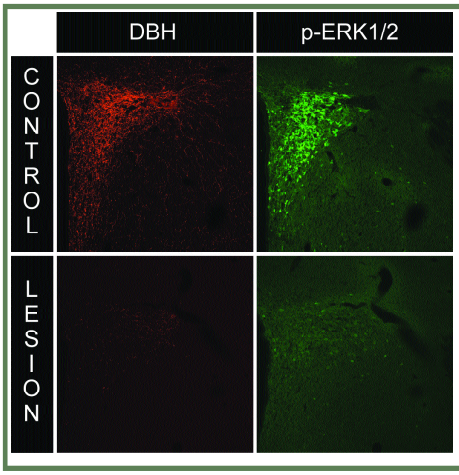

Fig 2. Rats receiving PVH injections of anti-DBH-SAP show a marked loss of catecholaminergic inputs, as seen from the drastic reductions in DBH immuno-staining (red signal, left column). This loss was accompanied by a loss of insulin-induced phospho-ERK1/2 signaling (green signal, right column).

Inside this issue:

Newsletter Highlights

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Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Spring is Here!

It's spring and the signs of new life in animals and plants is all around. This glorious scene took place outside Gangsta's (Dr. Lappi's) home in Del Mar. Two baby hummingbirds were lovingly attended by Mama Hummingbird for several weeks on the patio. Such an amazing site for a bird that so often is only seen in frantic motion.

These hummingbirds are called Anna's hummingbirds and are the most common hummingbird in southern California. Unlike most other hummingbirds, Anna's has a (minimal) song. According to Cornell experts, in the first half of the 20th century, the Anna's Hummingbird bred only in northern Baja California and southern California. The planting of exotic flowering trees provided nectar and nesting sites, and allowed the hummingbird to greatly expand its breeding range.

New Facility

TOP

The main laboratory has all the major equipment in a central location. Spectrophotometers, plate readers, liquid chromatography, luminometer and other shared equipment is easily accessible.

MIDDLE

Cytometry Research is our service facility which provides flow cytometry sorting and analysis. Other services include *in vitro* assays such as: antibody titration, cytotoxicity, cell line development, and cancer cell line screening.

BOTTOM LEFT

The Biomek provides the capability to do large-scale ELISA's and other assays with the computer-programmable robot.

BOTTOM RIGHT

ATS enjoys a great team spirit, including the fun times. Having space for a ping pong table out in the lab is a great feature to our new space. Competition runs high, but there's always a good time. Brian Russell and Matt Kohls hone their skills for the next match.

In February, Advanced Targeting Systems and Cytometry Research moved to new facilities in San Diego. Celebrating 15 years of service to the scientific community worldwide, this was a great change for this small company. In the previous location, expansion meant that the company was occupying two separate suites.

The new facility is located only a mile away from the old facility in Sorrento Valley, an area in the Golden Triangle of San Diego. This area is a biotechnology hub with The Salk Institute, UCSD, The Scripps Research Institute, Burnham Institute, Amylin, Vertex, and Neurocrine, just to mention a few.

This move was an important step in the advancement of the Company's capabilities to continue to provide superior customer service and new product development. Local customers have easy access to bring their cells in for assay and analysis. Customers worldwide will continue to receive quality, timely service for requests made directly to the San Diego office or to the European office in The Netherlands.

Call when you're in the area and receive a personal tour!

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Noradrenergic Neurons in the Locus Coeruleus Contribute to Neuropathic Pain

Brightwell JJ, Taylor BK
Neuroscience [Epub], 2009.

Noradrenergic neurons were eliminated with 5 μg intracerebroventricular injections of anti-DBH-SAP (Cat. #IT-03). Mouse IgG-SAP (Cat. #IT-18) was used as a control. Animals lesioned with anti-DBH-SAP displayed a reduction in behavioral signs of several kinds of allodynia.

Neuropeptide Y receptor-expressing dorsal horn neurons: Role in nocifensive reflex responses to heat and formalin

Wiley RG, Lemons LL, Kline RH
Neuroscience [Epub], 2008.

This work examines the effect of lumbar intrathecal administration of NPY-SAP (Cat. #IT-28), and the role of Y1 NPY receptor-expressing neurons (Y1R) in response to thermal and chemical stimulation. Rats received 500 ng or 750 ng intrathecal injections of NPY-SAP. Blank-SAP (Cat. #IT-21) was used as a control. Lesioned animals displayed a specific loss of Y1R in the dorsal horn, as well as reduced nocifensive reflex responses.

Cognitive Performances of Cholinergically Depleted Rats Following Chronic Donepezil Administration

Cutuli D, Foti F, Mandolesi L, De Bartolo P, Gelfo F, Federico F, Petrosini L
J Alzheimers Dis [Epub], 2009.

The authors examined whether donepezil could improve cognitive functions in rats with lesions of the cholinergic cells in the forebrain. Treated animals received 4 μg bilateral intracerebroventricular injections of 192-IgG-SAP (Cat. #IT-01), followed by treatment with donepezil or a control. Donepezil-treated animals performed significantly better than control animals.

Spinal NK-1 receptor-expressing neurons and descending pathways support fentanyl-induced pain hypersensitivity in a rat model of postoperative pain

Rivat C, Vera-Portocarrero LP, Ibrahim MM, Mata HP, Stagg NJ, De Felice M, Porreca F, Malan TP
Eur J Neurosci 29(4):727-737, 2009.

Opioids activate hyperalgesia and allodynia. The authors test the hypothesis that NK-1 receptor-containing ascending pathways play a role in sensitivity to fentanyl. Rats received an intrathecal injection of SP-SAP (Cat. #IT-07), and controls received saporin (Cat. #PR-01). The data indicate that these ascending pathways have a role in fentanyl-induced hyperalgesia.

Dependence of monocyte chemoattractant protein 1 induced hyperalgesia on the isolectin B4-binding protein versican

Bogen O, Dina OA, Gear RW, Levine JD
Neuroscience 159(2):780-786, 2009.

Monocyte chemoattractant protein 1 (MCP-1) is involved in generation of inflammatory and neuropathic pain, but the mechanisms underlying this involvement are not understood. Rats received 3.2 μg intrathecal injections of IB4-SAP (Cat. #IT-10). Ten days later the rats received intradermal MCP-1. Animals treated with IB4-SAP did not exhibit the mechanical hyperalgesia normally seen when treated with MCP-1.

Efficacy of a murine-p75-saporin immunotoxin for selective lesions of basal forebrain cholinergic neurons in mice

Nag N, Baxter MG, Berger-Sweeney JE
Neurosci Lett 452(3):247-251, 2009.

The authors tested a new version of mu p75-SAP (Cat. #IT-16) in mice. Mice received bilateral injections of 0.65 or 1.3 μg of immunotoxin into each lateral ventricle. Both amounts produced a complete loss of cholinergic neurons in the medial septum, while a dose-dependent loss of cholinergic neurons was seen in the nucleus basalis magnocellularis.

Developmental forebrain cholinergic lesion and environmental enrichment: behaviour, CA1 cytoarchitecture and neurogenesis

Frechette M, Rennie K, Pappas BA
Brain Res 1252:172-182, 2009.

The authors investigated the effect of neonatal cholinergic lesions on plasticity in the presence or absence of enrichment. Each lateral ventricle of 7 day-old rats received 300 ng of 192-IgG-SAP (Cat. #IT-01). Although the lesions did not attenuate neurobehavioral plasticity, there were several physiological changes that occurred despite the environmental enrichment.

(continued on page 4)

Please visit www.ATSbio.com to see a complete list of references.

Targeting Topics: Recent Scientific References

(continued from page 3)

Segregated populations of hippocampal principal CA1 neurons mediating conditioning and extinction of contextual fear

Tronson NC, Schrick C, Guzman YF, Huh KH, Srivastava DP, Penzes P, Guedea AL, Gao C, Radulovic J
J Neurosci 29(11):3387-3394, 2009.

This work examines what cell groups are responsible for controlling contextual fear. 180 ng of mu p75-SAP (Cat. #IT-16) was injected into the medial septum of mice. Saporin (Cat. #PR-01) was used as a control. In lesioned animals, fear extinction was lost along with the cholinergic input from the medial septum, while fear conditioning was left intact.

Cardiac damage after lesions of the nucleus tractus solitarii

Nayate A, Moore SA, Weiss R, Taktakishvili OM, Lin LH, Talman WT
Am J Physiol Regul Integr Comp Physiol 296(2):R272-279, 2009.

This work tested the hypothesis that nucleus tractus solitarii (NTS) lesions can lead to fatal cardiac arrhythmias and myocardial lesions. Rats received bilateral injections of 9.4 ng of SSP-SAP (Cat. #IT-11) into the dorsolateral and medial portions of the NTS. Lesioned animals displayed increased arterial blood pressure.

Neuroprotective effects of testosterone on the morphology and function of somatic motoneurons following the death of neighboring motoneurons

Little CM, Coons KD, Sengelaub DR
J Comp Neurol 512(3):359-372, 2009.

Atrophy of androgen-sensitive motoneurons due to proximity to damaged motoneurons can be attenuated by testosterone. This work examined whether typical motoneurons respond in the same way. Rats received 5-ng injections of CTB-SAP (Cat. #IT-14) that eliminated motoneurons innervating the vastus medialis muscle. Partial motoneuron depletion resulted in atrophy of the remaining quadriceps motoneurons; this was attenuated by the administration of testosterone.

Noradrenergic, but not cholinergic, deafferentation of prefrontal cortex impairs attentional set-shifting

McGaughy J, Ross RS, Eichenbaum H
Neuroscience 153(1):63-71, 2008.

Norepinephrine and acetylcholine are involved in the mediation of attention, however, it is not yet clear whether the roles of these molecules are unique. This work utilizes a specific task shown to dissociate the roles played by the dorsolateral prefrontal cortex and the orbitofrontal cortex in primates. Rats received 5-ng infusions of anti-DBH-SAP (Cat. #IT-03) or 192-IgG-SAP (Cat. #IT-01) into each hemisphere. The type of lesion had an effect on attentional shifts and reaction to irrelevant stimuli.

Sex differences in micro-opioid receptor expression in the rat midbrain periaqueductal gray are essential for eliciting sex differences in morphine analgesia

Loyd DR, Wang X, Murphy AZ
J Neurosci 28(52):14007-14017, 2008.

The authors test whether the periaqueductal gray (PAG), that contains a dense population of μ-opioid receptor (MOR)-expressing neurons, is sexually dimorphic. Rats were injected with 3 pmol of Dermorphin-SAP (Cat. #IT-12) into the PAG. Blank-SAP (Cat. #IT-21) was used as a control. Both behavioral and immunohistochemical evidence suggest that differential expression of MOR-expressing neurons in the PAG between male and female rats accounts for the difference in response to morphine.

Anxiety-like behavior is modulated by a discrete subpopulation of interneurons in the basolateral amygdala

Truitt WA, Johnson PL, Dietrich AD, Fitz SD, Shekhar A
Neuroscience [Epub], 2009.

It is thought that the basolateral nucleus of the amygdala (BL) is an anxiety regulator. The authors previously demonstrated that SSP-SAP (Cat. #IT-11) lesions of the BL increase anxiety-like behaviors in rats. Using a series of 6 bilateral injections of SSP-SAP (4 ng per injection), the NK-1 receptor-expressing cells of the BL are further characterized.

Neural regulation of ejaculation

Young B, Coolen L, McKenna K
J Sex Med 6 Suppl 3(229-233, 2009.

This review summarizes that a specific population of lumbar spinothalamic (LSt) cells plays in regulation of the ejaculatory response. One method to study these cells is the injection of SSP-SAP (Cat. #IT-11) into the LSt cells surrounding the central canal. Over 90% of these cells express the NK-1 receptor. This lesion significantly disrupts ejaculation without affecting mounts or intromissions.

Targeted ablation of cardiac sympathetic neurons reduces resting, reflex, and exercise-induced sympathetic activation in conscious rats

Lujan HL, Palani G, Chen Y, Peduzzi-Nelson J, DiCarlo SE
Am J Heart Circ Physiol
doi:10.1152/ajpheart.00095.2009

This work examines the capability of CTB-SAP (Cat. #IT-14) to eliminate cardiac sympathetic neurons. The right and left stellate ganglia of rats were each injected with 10 μg of CTB-SAP. Lesioned animals displayed physiological differences from controls, as well as specific reduction of numbers of neurons in the stellate ganglion and spinal cord.

Targeting Talk: Product Questions

by Dr. Douglas Lappi

Q: Could you please tell me if the Somatostatin 14 antibody (Cat. #AB-04) will also pick up the Somatostatin 28 residue?

A: Yes, it will, because they share the sequence of SS14. However, the Somatostatin-28 antibody (Cat. #AB-05) will not see Somatostatin-14.

* * * * * * *

Q: Could you confirm if Anti-Conjugated Caprylic Acid (Cat. #AB-T084) can detect Caprylic acid unconjugated with BSA or only the protein conjugated with BSA or with another carrier protein?

A: This antibody does not need BSA to be present or conjugated in order for it to bind Caprylic acid. However, it DOES need to be used in the presence of gluteraldehyde in order to create the proper epitope for the antibody to recognize the Caprylic acid.

Q: We are using SSP-SAP (Cat. #IT-11) to lesion NK-1r-bearing neurons. I have the conjugate diluted in solution and was wondering whether or not it is okay to leave it out at room temperature overnight? I would like to use an aliquot over a period of two days. Also, would it be okay to combine it the next day to a new, thawed aliquot?

A: We suggest, instead of leaving material out at room temperature, that you store at 4°C over the two days. Yes, you can combine samples.

* * * * * * *

Q: Regarding your HRP-labeled Antibody to p53 (Cat. #AB-236), your data sheet states the antigen was 15-40 a.a. Does this a.a. count from N-terminus?

A: There was an error on the data sheet which has since been corrected. The AB-236 immunogen is a KLH-conjugated peptide corresponding to amino acids 6-45. The numbering does start from the N-terminus.

Upcoming Events

Amer Assoc of Immunologists
May 8-12
Seattle, WA
Booth #208

Society for Neuroscience
October 17-21
Chicago, IL
Booth #TBD

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: TETRAMER
COGNATE
MECHANISM
INFECT
SELECT

Answer: She looked for . . .CHANGE

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

WINNERS: Mark Charman, Dalhousie Univ, Halifax CANADA * Kim Van Vliet, Univ Florida, Gainesville, FL * Jason Meisner, Dalhousie Univ, Halifax CANADA

NEW! Solve the Teaser online at:
http://www.atsbio.com/news/09q2_teaser.html

Featured Antibodies

NGFr (mu p75) Rabbit Polyclonal

Cat. #AB-N01, 100 µl or affinity-purified Cat. #AB-N01AP, 50 µg

Recognizes: p75NTR in mouse.
Applications: immunohistochemistry (frozen or paraffin-embedded cells and tissue (1:150), immunoprecipitation, immunoblotting (1:2,000), flow cytometry (1:1,000), blocks the function of NGFr (1:1,000).

NGFr (ME20.4, p75) Mouse Monoclonal

Cat. #AB-N07, 100 µg

Recognizes: p75NTR (low affinity neurotrophin receptor) in human, primate, rabbit, sheep, dog, cat, and pig.
Applications: flow cytometry (1:100), immunoprecipitation, immunohistochemistry (frozen), electron microscopy (1:200), immunocytochemistry (10 ng/ml), RIA.

Angiotensin II receptor rabbit polyclonal

Cat. #AB-N28AP 50 µg (affinity purified)

Recognizes: Angiotensin II type 2 receptor (AT-2) in rat.
Applications: immunolabeling (1:500). immunohistochemistry (paraffin).

Deletion of Catecholaminergic Neurons by Anti-DBH-Saporin

(continued from page 1)

CRH neuroendocrine neurons under these conditions is deduced, in part, by observing changes in the expression of various cellular markers within these neurons in control vs. stress conditions in laboratory rats. For example, we have shown that systemic insulin or 2-DG injection rapidly elevates levels of the phosphorylated forms of MAP kinases ERK1 and/or ERK2[1,2]. The phospho-ERK1/2 staining appears to be induced selectively by these challenges, as little to no phospho-ERK1/2 appears in the CRH neuroendocrine neurons under basal conditions (Fig. 1).

We employed the use of anti-DBH-SAP (Cat. #IT-03) injections to determine whether this phospho-ERK1/2 response requires intact catecholaminergic afferents originating in the hindbrain. Rats given PVH microinjections of anti-dopamine-beta-hydroxylase-saporin conjugate (anti-DBH-SAP) or mouse IgG-saporin control conjugate (Cat. #IT-18) received either normal 0.9% saline vehicle or insulin (2 U/kg, i.v.) and received lethal doses of intravenous anesthesia 30 min later. Brains were processed for DBH and phospho-ERK1/2 immunocytochemistry. Relative to rats receiving sham lesions (injections of control conjugate), anti-DBH-SAP-treated animals displayed a marked loss of DBH immunostaining, indicative of a pronounced loss of catecholaminergic fibers (Fig. 2). This loss was accompanied by loss of the phospho-ERK1/2 response to insulin or 2-DG within these neurons, as evident from the marked reductions of phospho-ERK1/2 immunostaining observed in these animals (Fig. 2, page 1). Additionally, hindbrain cell groups in lesioned rats displayed frank loss of DBH staining, indicative of true cell loss in these hindbrain groups as a result of the saporin lesions (Fig. 3).

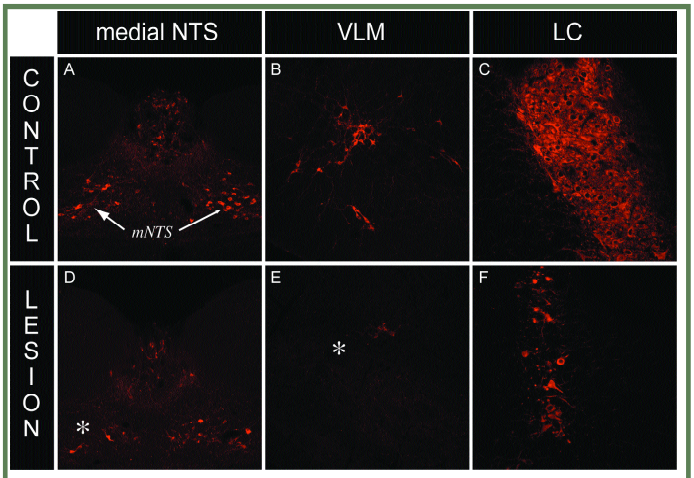


Fig 3. Confirmation of lesion efficacy: The loss of DBH staining in the paraventricular hypothalamus was accompanied by cell loss in hindbrain catecholaminergic cell groups expressing DBH (including the medial subnucleus of the nucleus of the solitary tract (mNTS), the ventrolateral medulla (VLM), and the locus coeruleus (LC); compare D, E and F with A, B, and C, respectively), indicating that the lost DBH+ inputs were from these neurons. Asterisks indicate areas showing cell loss as compared to corresponding regions in control animals.

A characteristic response of CRH neuroendocrine neurons to various homeostatic challenges is the initiation of CRH gene transcription, which is thought to be controlled, in part, by the activation (phosphorylation) of the transcription factor, CREB (cyclic AMP response element binding protein). Indeed, we observed phospho-CREB levels to increase markedly in response to insulin and 2-DG in PVH neurons. To ascertain what happens to CREB activation in CRH neuroendocrine neurons in rats receiving anti-DBH-SAP lesions, we performed dual immunocytochemistry for both markers in lesioned rats. As shown in Fig. 4, lesioned animals displayed marked reductions in phospho-CREB, in line with the reductions observed in phospho-ERK1/2 levels.

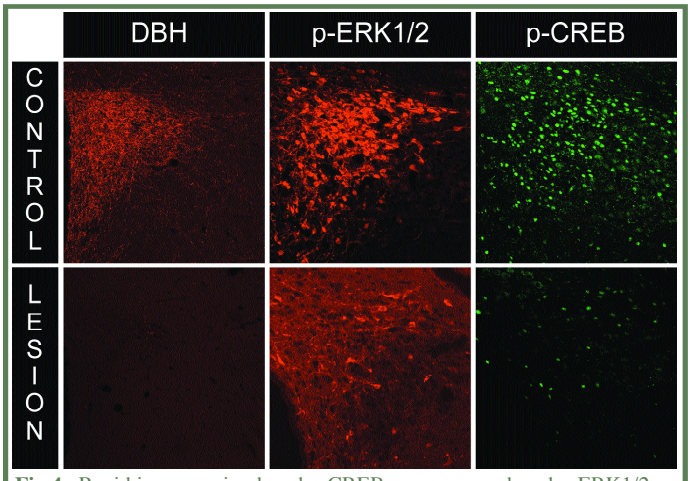


Fig 4. Rapid increases in phospho-CREB accompany phospho-ERK1/2 elevations in the paraventricular hypothalamus of intact (non-lesioned) animals receiving 2-deoxy-D-glucose (2-DG). Loss of DBH is associated with disruptions in both 2-DG-induced phospho-ERK1/2 and phospho-CREB signaling, demonstrating their dependence on catecholaminergic input.

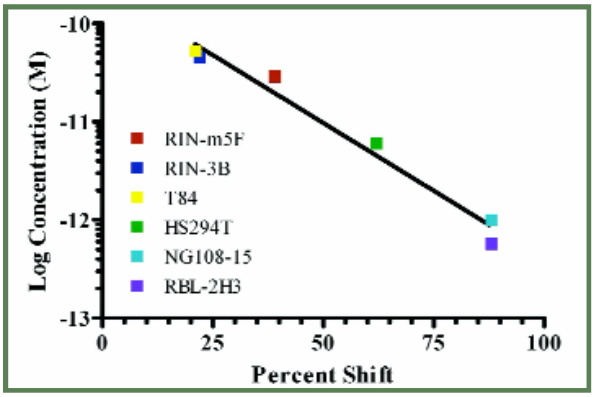
Collectively, our data demonstrate that rats mount CRH neuroendocrine responses to glycemic challenges in a manner that requires intact catecholaminergic afferents from the hindbrain. Both phospho-ERK1/2 and phospho-CREB signals appear to rely on signals propagating along these catecholaminergic pathways. The targeting of this afferent system using saporin-based immunotoxin conjugates has proven to be a valuable technique for probing the afferent circuitry of this important physiological system.

References:

1. Khan AM, Watts AG. 2004. Intravenous 2-deoxy-D-glucose injection rapidly elevates levels of the phosphorylated forms of p44/42 mitogen-activated protein kinases (extracellularly regulated kinases 1/2) in rat hypothalamic parvicellular paraventricular neurons. *Endocrinology* 145(1):351-359.
2. Khan AM, Ponzio TA, Sanchez-Watts G, Stanley BG, Hatton GI, Watts AG. 2007. Catecholaminergic control of mitogen-activated protein kinase signaling in paraventricular neuroendocrine neurons *in vivo* and *in vitro*: A proposed role during glycemic challenges. *The Journal of Neuroscience* 27(27):7344-7360.

Targeting Tools: Featured Products

CTB-SAP



The percent shift as determined by flow cytometry staining with CTB-FITC was plotted against the log concentration of the ED_{50} in moles/L of CTB-SAP for six cell lines. RIN 3B and RIN-m5F are rat insulinoma cell lines; T84 is a human colon carcinoma cell line; NG108-15 is a rat-mouse hybrid neuroblastoma/glioma cell line; RBL-2H3 is a rat basophilic leukemic cell line; HS294T is a human melanoma cell line. The plotted data show a distinct correlation between the number of CTB binding sites and the ED_{50} of CTB-SAP. Data were analyzed by Prism (GraphPad).

CTB-SAP is a conjugate between the cell-binding component of cholera toxin (the B chain) and saporin. CTB binds to GM1 (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.

As seen in the figure, the cytotoxicity is dependent on the quantity of GM1 on the cell surface, from little effect at zero expression to pM ED_{50} at high expression. The use of this conjugate has recently been the subject of several studies, and is providing use to researchers in many fields.

Hug-M-ZAP NEW!

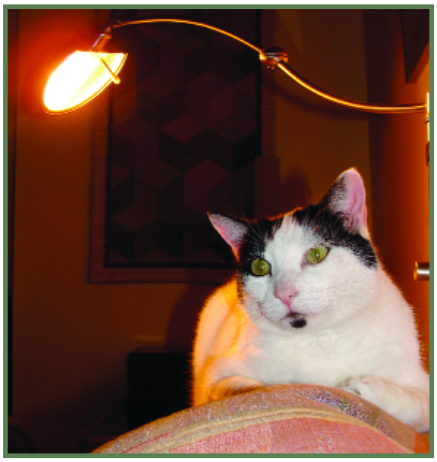
This secondary conjugate (molecular weight 210 kDa) recognizes YOUR human monoclonal IgM antibody. Hug-M-ZAP (IT-43) is a chemical conjugate of affinity-purified goat anti-human IgM and the ribosome-inactivating protein, saporin. Hug-M-ZAP uses your primary human monoclonal IgM antibody to target and eliminate cells and can be used to evaluate the potential of a primary antibody to internalize.

Also try our other Secondary Antibody Conjugates:

Anti-M-ZAP (IT-30)	Mab-ZAP (IT-04)
Goat-ZAP (IT-36)	Rab-ZAP (IT-05)
Hum-ZAP (IT-22)	Rat-ZAP (IT-26)

Summer's almost here.

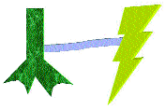
Gotta work on my tan!



Targeting Technology

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

Choose an ANTIBODY§ specific to your cell type.



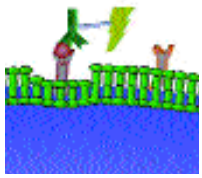
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

§ or anything recognized on the cell surface and internalized.

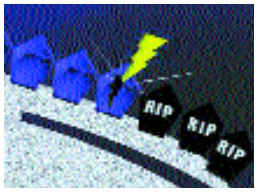
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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, one letter to each block, to solve the puzzle.

ASKEIN ☐☐☐☐☐☐

STRIPDU ☐☐☐☐☐☐☐

GLEANBIL ☐☐☐☐☐☐☐☐

GATERULE ☐☐☐☐☐☐☐☐

VATICANITO ☐☐☐☐☐☐☐☐☐☐

How the intern rated the two-week lab study.

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

ANSWER: It was his best . . . ☐☐☐☐☐☐ ☐☐☐☐☐

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, 2009
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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Jul-Aug-Sep 2009
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Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Depletion of Microglia by Mac-1-SAP in Mouse Hippocampal Slice Cultures Enhances Ischemia-Like Neurodegeneration

Contributed by Maria Montero,[a] Berta González,[b] Jens Zimmer[a]
[a]*Anatomy and Neurobiology, University of Southern Denmark, Denmark*
[b]*Histology Unit, Autonomous University of Barcelona, Spain*

Microglial cells contribute about 12% of the cells in the brain, acting as "biosensors" for homeostatic regulation in normal and pathological conditions.[3,13] Resting microglial cells in the adult brain have thin ramified processes, but transform into a non-phagocytic activated phenotype, or a phagocytic activated phenotype when activated.[14] Upon activation, structural changes towards an amoeboid appearance occur with transformation of the normal fine processes into short coarser processes[4,14] in parallel with upregulation of normal markers like Mac-1,[12] MHC class I and II molecules[5] and secretion of cytokines like interleukin-1beta (IL-1β),[2] transforming growth factor beta-1 (TGF-β1)[6] and tumor necrosis factor alpha (TNF-α).[1] The reactions to injury also include a drastic increase in the number of microglial cells at the lesion site, due to migration and local proliferation.[10] However, it is still not clear to what extent or under which conditions activated forms of microglia exert positive or negative effects on neuronal survival.[7] Experimental depletion of microglial cells, before application of a standardized neurodegenerative insult, would, however, help clarify the protective or degenerative role of microglial cells.

Depletion of microglial cells in mouse brain hippocampal slice cultures was successfully achieved by adding a microglia-targeting toxin, Mac-1-SAP (a conjugate of saporin and a CD11b antibody) to the culture medium for 7 days (Fig. 1).[8]

For experimental testing of the effects of microglia depletion on ischemia-induced neurodegeneration, Mac-1-SAP treated and corresponding untreated hippocampal slice cultures were subjected to standardized 30-min oxygen-glucose deprivation (OGD), mimicking transient cerebral ischemia and preferentially causing the more susceptible CA1 pyramidal cells to degenerate. The resulting neuronal cell death was quantified by densitometric measurements of the cellular uptake of the fluorescent intercalating agent propidium iodide (PI) in the CA1 area[11] (Fig. 2). Other Mac-1-SAP treated and control cultures were stained immunohistochemically for neuronal, astroglial and microglial markers 1, 7 or 14 days after OGD (Fig. 3).

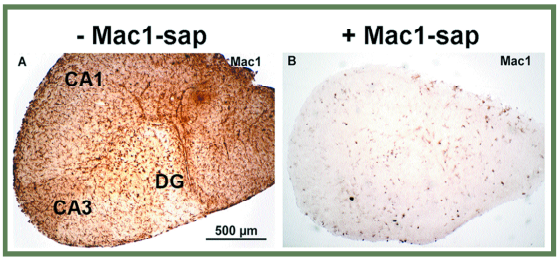


Fig. 1. A: Microglial cells in mouse hippocampal slice culture, visualized by immunohistochemical staining for Mac-1/CD11b. **B:** Immunotoxic depletion of microglial cells in mouse hippocampal slice culture treated with Mac-1-SAP for 7 days. Following this treatment almost all microglial cells are killed. Only a few remaining cells with abnormal morphology express the microglial marker Mac-1. Corresponding observations were made in slice cultures stained for other microglial markers such as tomato lectin (not shown). Scale bar valid for both A and B.

Twenty-four hours after OGD, the Mac-1-SAP treated, microglia-depleted hippocampal slice cultures displayed, in comparison to correspondingly OGD-exposed control cultures, a significant increase in CA1 pyramidal cell death, as *(continued on page 6)*

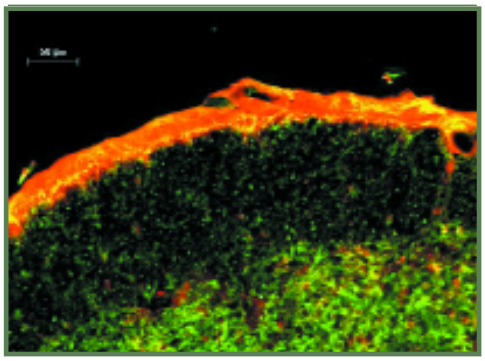
SBIR Grant Funded: *Selective Activation in Neuronal Populations*

ATS has been awarded a Phase I SBIR grant to develop a new line of products. Dr. Douglas Lappi is the Principal Investigator and Brian Russell is the Lead Scientist on the project. Molecules targeted towards cell surface markers have been used for years to identify specific cell types. It has been demonstrated over the course of the past decade that biologically-active molecules, when attached to these cell surface-binding molecules, can be delivered in a specific manner, utilizing the tendency of a bound receptor to be internalized. Frequently, delivery of biologically-active molecules has resulted in cell death or inhibition. It is proposed in this project to direct this technology toward specific neuronal populations with the intention of activating these cells temporarily, thereby increasing neurotransmission.

The proof of concept will include synthesis of a conjugate of an antibody to the mouse low-affinity neurotrophin receptor (p75; Cat. #AB-N01AP) and the enzymatic A1 fragment of cholera toxin (CTA1), and examination of the effects on neuronal cells that express the p75 receptor. In previous trials with CTA attached to Substance P, Caudle *et al*. have shown the conjugate to be useful for stimulating NK1 receptor-expressing neurons in the dorsal horn. SP-CTA (Cat. #IT-39) has been tested both *in vivo* and *in vitro* and provided excellent results in both venues.

The success of this funded project would revolutionize targeted conjugate technology. The ability for researchers to study the effects of an activated or amplified neuronal system, rather than the results of a neuronal deficit through transgenics or immunotoxins, would allow for greater understanding of the neuronal function and physiology. The application of proven ATS research tools could significantly enhance the possibility of success in therapeutic applications for the treatment of neuropsychiatric and other maladies.

Caudle R.M., Mannes AJ, Keller J, Perez FM, Suckow SK, Neubert JK. Sensitization of spinal cord nociceptive neurons with a conjugate of substance P and cholera toxin. *BMC Neurosci*, 8:30 (2007).



SP-CTA (10 μg) injected intracisternally into rats via a percutaneous puncture under isoflurane anesthesia. 1h later animals were euthanized and sections (20 μm) of the brain stem and cervical spinal cord prepared for immunohistochemistry. Immunofluorescence co-labeling for the NK1 receptor (Green) and for the A subunit of cholera toxin (Red) was performed. The cholera toxin subunit was found only in neurons co-labeled with NK1 receptor.

New Antibodies -

BDNF	Mouse Anti Human Brain-Derived Neurotrophic Factor
c-Myc	Mouse Anti Human c-Myc
CD11a	Rat Anti Mouse CD11a
CD1A	Mouse Anti Human CD1A (T6, LEU6)
CD2	Mouse Anti Human CD2 (T11, LFA-2)
CD20	Recombinant Anti Human CD20 Antibody
CD3	Mouse Anti Human CD3
CD4	Mouse Anti Human CD4
CD5	Mouse Anti Human CD5
CD62E	Mouse Anti Human E-Selectin
CD8	Mouse Anti Human CD8
CD80	Rat Anti Mouse CD80
EGF	Mouse Anti Human Epidermal Growth Factor
EPO	Mouse Anti Human Erythropoietin
GMCSF	Mouse Anti Human Granulocyte Macrophage-Colony Stimulating Factor
IFN-a Neut	Mouse Anti Human Interferon-alpha Neutralizing
IFN-b	Mouse Anti Human Interferon-beta
IFN-g	Mouse Anti Human Interferon-gamma
IL-10	Mouse Anti Human Interleukin-10
IL-15	Mouse Anti Human Interleukin-15
IL-2	Mouse Anti Human Interleukin-2
IL-2r	Mouse Anti Human Interleukin-2 receptor
IL-3	Mouse Anti Human Interleukin-3
IL-4	Mouse Anti Human Interleukin-4
IL-6	Mouse Anti Human Interleukin-6
IL-7	Mouse Anti Human Interleukin-7
IL-8	Mouse Anti Human Interleukin-8
Leptin	Mouse Anti Human Leptin
Myc	Mouse Anti Myc
NT-4	Mouse Anti Human Neurotrophin-4
p53	Recombinant Anti p53 scFv (Cat. #AB-301)
TGF-beta	Mouse Anti Human Transforming Growth Factor-beta
TNF-a	Mouse Anti Human Tumor Necrosis Factor-alpha
VEGF	Mouse Anti Human Vascular Endothelial Growth Factor

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Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Photostimulation of retrotrapezoid nucleus phox2b-expressing neurons in vivo produces long-lasting activation of breathing in rats

Abbott SB, Stornetta RL, Fortuna MG, Depuy SD, West GH, Harris TE, Guyenet PG
J Neurosci 29(18):5806-5819, 2009.

The retrotrapezoid nucleus (RTN) contains a subpopulation of cells that are thought to function as central respiratory chemoreceptors. The authors used bilateral 22-ng injections of anti-DBH-SAP (Cat. #IT-03) into the lateral horn of the second thoracic segment to investigate this hypothesis. Coupled with data generated by lentivirus-driven transgenic expression of a light-activated cationic channel, it is demonstrated that noncatecholaminergic neurons in the RTN function as central respiratory chemoreceptors.

Anti-amnesic properties of (+/-)-PPCC, a novel sigma receptor ligand, on cognitive dysfunction induced by selective cholinergic lesion in rats

Antonini V, Prezzavento O, Coradazzi M, Marrazzo A, Ronsisvalle S, Arena E, Leanza G
J Neurochem 109(3):744-754, 2009.

Sigma-1 receptors are found throughout the central nervous system, and are thought to be a target for regenerative therapy in Alzheimer's disease. Rats received 3.0 μg or 5.0 μg of 192-IgG-SAP (Cat. #IT-01) injected intracerebroventricularly. The lesioned animals displayed dose-dependent deficits in water maze performance. Treatment with the sigma-1 receptor agonist (±)-PPCC significantly improved both reference and working memory performance in treated animals, indicating that (±)-PPCC-mediated positive effects are probably a function of the sigma-1 receptor.

Cholinergic deafferentation of the neocortex using 192 IgG-saporin impairs feature binding in rats

Botly LC, De Rosa E
J Neurosci 29(13):4120-4130, 2009.

It has been hypothesized that the nucleus basalis magnocellularis (NBM) is the source of cholinergic input to the neocortex that is responsible for incorporating different features of an object into a unified neural representation of said object. Rats received 0.04-μg bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the NBM. In lesioned animals modes of learning requiring feature binding were impaired, while processes not using feature binding were left intact.

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 [Epub], 2009.

In this work the authors investigated the link between connections carried by the fornix and cholinergic input to the inferotemporal cortex in scene learning. Monkeys received 56-64 0.02-μg injections of ME20.4-SAP (Cat. #IT-15) into the inferotemporal cortex, and entorhinal cortices. There was a marked impairment in memory for lesioned animals that also received a fornix transection, indicating a synergistic interaction between connections carried by the fornix and cholinergic input to the inferotemporal cortex for episodic memory.

Substance P neurotransmission and violent aggression: the role of tachykinin NK1 receptors in the hypothalamic attack area

Halasz J, Zelena D, Toth M, Tulogdi A, Mikics E, Haller J
Eur J Pharmacol 611(1-3):35-43, 2009.

Stimulation of the hypothalamic attack area elicits biting attacks in rats. The authors eliminated NK1 receptor-expressing neurons in this area with bilateral 6.25-ng injections of SP-SAP (Cat. #IT-07). Violent attacks were dramatically reduced while milder forms of aggression remained unchanged, indicating that these two forms of aggression are controlled via different pathways. Lesioned animals also displayed reduced anxiety-like behavior in the elevated plus-maze, suggesting a connection between the hypothalamic attack area and brain areas controlling anxiety.

Partial ablation of mu-opioid receptor rich striosomes produces deficits on a motor-skill learning task

Lawhorn C, Smith DM, Brown LL
Neuroscience [Epub], 2009.

The functional role of basal ganglia striosomes is not well understood. In order to examine these cells in the context of motor behavior the authors injected 8.5 ng of dermorphin-SAP (Cat. #IT-12) into several areas of the striatum of mice (saporin, Cat. #PR-01, was used as a control). The animals were then evaluated in complex motor tasks involving the use of striatal circuitry. Animals receiving dermorphin-SAP showed deficits in specific motor tasks corresponding to the extent of the lesion.

Targeted ablation of cardiac sympathetic neurons reduces resting, reflex and exercise-induced sympathetic activation in conscious rats

Lujan HL, Palani G, Chen Y, Peduzzi JD, Dicarlo SE
Am J Physiol Heart Circ Physiol 296(5):H1305-1311, 2009.

Excessive sympathetic activity contributes to most cardiovascular diseases. Thoracic sympathectomy is a non-specific treatment that can alleviate some sympathetic activity, but produces undesirable side effects. The authors lesioned a subset of sympathetic preganglionic neurons with 10 μg of CTB-SAP (Cat. #IT-14) into the left and right stellate ganglion of rats. Treated animals displayed several types of reduced cardiac sympathetic neuronal activity indicating that this may be a useful approach for treating these types of conditions.

Please visit www.ATSbio.com to see a complete list of references.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Neuroprotective effects of the anti-inflammatory compound triflusal on ischemia-like neurodegeneration in mouse hippocampal slice cultures occur independent of microglia

Montero Dominguez M, Gonzalez B, Zimmer J
Exp Neurol 218(1):11-23, 2009.

In this work the authors looked to clarify the role of microglia in an experimental stroke model. Hippocampal slices were subject to oxygen-glucose deprivation to establish the stroke model. Slices were exposed to 1.3 nM Mac-1-SAP (Cat. #IT-06) for 7 days prior to the experiments. This treatment depleted virtually all of the microglia.

See Cover Story for details.

Central chemoreception is a complex system function that involves multiple brain stem sites

Nattie E, Li A
J Appl Physiol 106(4):1464-1466, 2009.

This short review discusses central chemoreception and the different neuronal subtypes that play roles in this process. The use of anti-SERT-SAP (Cat. #IT-23) and anti-DBH-SAP (Cat. #IT-03) is mentioned in the context of how the loss of each of these cell types affects CO_2 response in rats.

Neurotrophic signaling molecules associated with cholinergic damage in young and aged rats: Environmental enrichment as potential therapeutic agent

Paban V, Chambon C, Manrique C, Touzet C, Alescio-Lautier B
Neurobiol Aging [Epub], 2009.

This study examined the potential of long-term environmental enrichment as a therapeutic agent for cholinergic damage. Rats received bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum (37.5 ng per side) and nucleus basalis magnocellularis (75 ng per side). Through the use of cDNA macroarrays the authors associated the therapeutic effects of environmental enrichment with downregulation of gene expression associated with certain cell processes, and upregulation of gene expression associated with signal transduction.

A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response

Radley JJ, Gosselink KL, Sawchenko PE
J Neurosci 29(22):7330-7340, 2009.

GABAergic neurons have been implicated in the negative regulation of the hypothalamic-pituitary-adrenal axis (HPA). In order to clarify GABAergic input to the paraventricular hypothalamic nucleus the authors injected 0.23 μg of GAT1-SAP (Cat. #IT-32) into the anterior bed nucleus of the stria terminalis. Both unilateral and bilateral injections were used. Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The data indicate that the GABAergic neuronal population functions as proximate mediator of HPA-inhibitory limbic influences.

Neuropathic pain is maintained by brainstem neurons co-expressing opioid and cholecystokinin receptors

Zhang W, Gardell S, Zhang D, Xie JY, Agnes RS, Badghisi H, Hruby VJ, Rance N, Ossipov MH, Vanderah TW, Porreca F, Lai J
Brain 132(Pt 3):778-787, 2009.

A subpopulation of rostral ventromedial medulla (RVM) neurons express both the mu opioid receptor (MOR) and the cholecystokinin type 2 receptor (CCK_2). The authors tested the hypothesis that co-expression of these receptors is necessary for maintaining neuropathic pain. Rats received 50-ng bilateral injections of dermorphin-SAP (Cat. #IT-12), CCK-SAP (Cat. #IT-31), or the control (saporin alone, Cat. #PR-01) into the RVM. The data indicate that neurons co-expressing these receptors facilitate pain and can be directly activated by CCK input to the RVM.

The basal forebrain cholinergic system is required specifically for behaviorally mediated cortical map plasticity

Ramanathan D, Tuszynski MH, Conner JM
J Neurosci 29(18):5992-6000, 2009.

In this work the authors examined what types of neuronal plasticity require the cholinergic system. Selective depletion of the basal forebrain cholinergic system was accomplished by bilateral 112-ng and 75-ng injections of 192-IgG-SAP (Cat. #IT-01) into the nucleus basalis magnocellularis/ substantia inominata. The results indicate a linkage between cholinergic mechanisms and distinct forms of cortical plasticity, supporting the role of the forebrain cholinergic system in modulating plasticity associated with behavioral experience.

Ablation of least shrew central neurokinin NK1 receptors reduces GR73632-induced vomiting

Ray AP, Chebolu S, Ramirez J, Darmani NA
Behav Neurosci 123(3):701-706, 2009.

In this work the authors investigated the role of central and peripheral nervous systems components that mediate the emetic reflex. Least shrews received a 600-ng injection of SSP-SAP (Cat. #IT-11) into the lateral ventricle. Some animals also received a 4.8-μg intraperitoneal injection of SSP-SAP. Blank-SAP (Cat. #IT-21) and unconjugated saporin (Cat. #PR-01) were used as controls. Lesioned animals displayed reduced emesis, but the data indicate that a minor peripheral nervous system component is also present.

Neuropeptide Y receptor-expressing dorsal horn neurons: role in nocifensive reflex responses to heat and formalin

Wiley RG, Lemons LL, Kline RH 4th
Neuroscience 161(1):139-147, 2009.

In order to clarify the role of dorsal horn Y1 neuropeptide Y receptors (Y1R) in nocifensive responses to aversive stimuli, the authors injected 500-750 ng of NPY-SAP (Cat. #IT-28) into the intrathecal space of rats. Blank-SAP (Cat. #IT-21) was used as a control. Lesioned animals displayed specific loss of Y1R and increased latencies on several reflex response tests indicating a role for Y1R in nociception.

Targeting Talk: Product Questions

by Dr. Douglas Lappi

Q: We're interested in trying out your melanopsin antibody (Cat. #AB-N38) using immunohisto-chemistry in mouse retina. Do you have a recommended protocol?

A: Yes. The following protocol has been utilized successfully with anti-melanopsin.[1] This protocol is also available on our website. Just click on "Protocols" on our Home Page. ***www.ATSbio.com***

[1]Panda S. *et al*. 2002. Melanopsin (Opn4) requirement for normal light- induced circadian phase shifting. Science 298(5601):2213-2216.

* * * * * * *

Immunohistochemistry Protocol (AB-N38)

Primary Antibody

****200 µl/slide****

• Xylene 2X 5 min
• 100% EtOH 2X 5 min
• 95% EtOH 2X 5 min
• 70% EtOH 5 min
• PBS 10 min

****PFA Only Starts****

• Citric acid 2 min in microwave
• Cool 5-20 min room temperature
• PBS 5 min

****PFA Only Ends****

• Block with 5% serum/PBS 1 hr in humidified chamber
• Primary ab in blocking solution overnight @ 4°C in humidified chamber

Secondary Antibody

****Keep dark at all times****
****Use different wavelengths for multiple labeling****

• PBS 10 min
• Secondary ab 1:200 in PBS according to species 1 hr
• PBS 10 min
• DAPI 1:200 in PBS 30 sec
• PBS 10 min
• Vectashield

Upcoming Events

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Society for Neuroscience
October 17-21
Chicago, IL
Booth #619

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The solution to the puzzle was:

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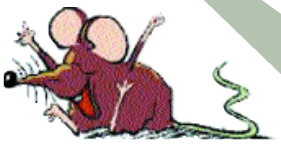
ACTIVATION



Answer: It was his best . . .SPRING BREAK

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Depletion of Microglia by Mac-1-SAP Enhances Ischemia-Like Neurodegeneration

(continued from page 1) monitored by cellular PI uptake (Fig. 2), as well as increased astroglial reactivity. At the same time point, the OGD lesioning induced an increase in Mac-1-positive cells in the microglia-depleted cultures, meaning that the few visible Mac-1 cells remaining after the immunotoxic treatment (and possible additional ones with staining below detection level) did, as normally, respond to the ischemia-like insult by stronger staining for microglial markers (Fig. 3). Using BrdU-labeling of dividing cells we also demonstrated lesion-induced proliferation of the few remaining microglial cells.[8] At 7 and 14 days after OGD the differences between Mac-1-SAP treated and non-treated cultures remained noticeable in terms of more neuron loss in cultures deprived of microglia, while the astroglial reactivity seemed to even out. During the same period more microglial cells appeared in the originally deprived cultures at the same time as the appearance of the cells normalized (Fig. 3 G, H). Such reoccurrence over time has also been noted for depleted cultures left to survive for an additional 2-3 weeks.

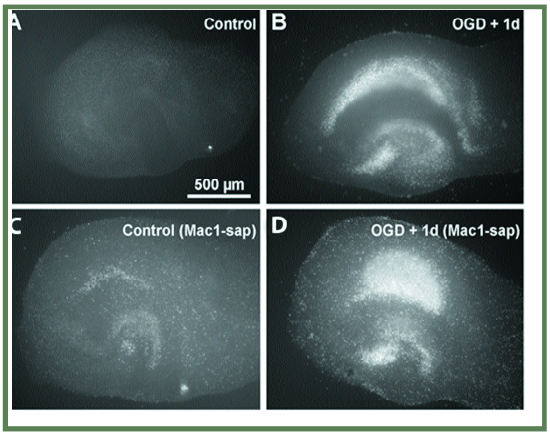
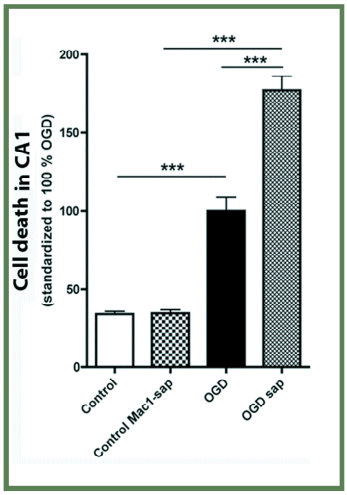


Fig. 2. Upper panel: Fluorescent micrographs of distribution and density of cellular uptake of PI in control (A, C) and OGD-lesioned mouse hippocampal slice cultures (B, D) with normal microglial content (A, B) and prelesional depletion of microglia (C, D). The OGD-induced cell death primarily affects CA1 pyramidal cells (CA1). **Lower panel:** Densitometric measurements of the cellular uptake of PI in CA1 pyramidal layer of untreated and Mac-1-SAP treated slice cultures as well as corresponding cultures 24 hours after 30 min. of OGD. Data are shown as a percentage of PI uptake standardized to OGD (n = 11-18) (*** $p < 0.001$).



Conclusions: (1) Exposure of mouse hippocampal slice cultures to Mac-1-SAP efficiently eliminates almost all microglial cells present in the cultures. (2) Mac-1-SAP treatment does not by itself induce increased neuronal cell death as monitored by cellular uptake of PI. (3) When subjected to an ischemia-like transient oxygen-glucose deprivation (OGD), Mac-1-SAP treated cultures displayed an increase in CA1 pyramidal cell death, as compared to non-treated OGD-lesioned cultures.

Perspectives: Brain slice cultures are easily accessible for microscopical inspection and addition of compounds and, as such, widely used as experimental models for CNS injury and disease and for screening of neurotoxic and neurotrophic compounds. Efficient and controlled depletion of microglial cells by Mac-1-SAP in slice cultures is a valuable tool for studies of microglial interactions with other cell types in the CNS, and microglia-mediated actions of, for example, anti-inflammatory compounds.[8]

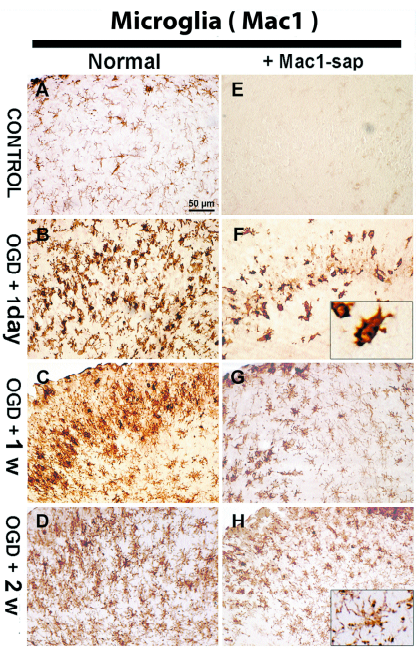


Fig. 3. Microglial depletion and reactions to 30 min OGD in CA1 area of mouse hippocampal slice cultures, visualized by Mac-1 immunohistochemistry. Treatment of 1 week old slice cultures with the Mac-1-SAP complex for 1 week dramatically reduced the number of microglial cells (A and E). One day after OGD, the number of reactive, Mac1-positive microglial cells increased in both normal (non-depleted) (B) and depleted cultures (F), followed by a normalization in morphology and staining density over the next 2 weeks, which also included the originally depleted slice cultures (E, F, G, H).

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Acknowledgments: Figures were published in *Brain Research*, Montero M., González B., Zimmer J. Immunotoxic depletion of microglia in mouse hippocampal slice cultures enhances ischemia-like neurodegeneration, in press. Copyright Elsevier, 2009.

Targeting Tools: Featured Products

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Immunogen: synthetic acrolein conjugated to bovine serum albumin
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Angiotensin II receptor (AT-1Ar) affinity-purified Rabbit Polyclonal (Cat. #AB-N25AP, 50 µg)

Specificity: Recognizes the AT-1A isoform of the Angiotensin II type 1 receptor in rat.
Usage: Applications include immunolabeling (1:500).

Angiotensin II receptor (AT-1r) affinity-purified Rabbit Polyclonal (Cat. #AB-N27AP, 50 µg)

Specificity: Recognizes isoforms AT-1A and AT-1B of the Angiotensin II type 1 receptor (AT-1r) in rat.
Usage: Applications include immunohistochemistry (immunoperoxidase electron microscopy, 1:500).

Angiotensin II receptor (AT-2r) affinity-purified Rabbit Polyclonal (Cat. #AB-N28AP, 50 µg)

Specificity: Recognizes the Angiotensin II type 2 receptor (AT-2) in rat.
Usage: Applications include immunolabeling (1:500).

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(through September 30, 2009)

Anthranilic Acid Rabbit Polyclonal (Cat. #AB-T155, 50 µl)

Immunogen: Synthetic Anthranilic Acid conjugated to bovine serum albumin
Usage: Applications include ELISA (1/5,000-1/10,000) and immunohistochemistry / immunocytochemistry (frozen sections).

Dopamine Mouse Monoclonal (Cat. #AB-T11, 50 µl)

Immunogen: Synthetic dopamine conjugated to bovine serum albumin
Usage: Applications include ELISA (1/1,000-1/5,000), immunocytochemistry, immunohistochemistry (1/1,000-1/5,000), immunoblotting (western blot, 1/1,000-1/2,000).

Dopamine Rabbit Polyclonal (Cat. #AB-T07, 50 µl)

Immunogen: Synthetic dopamine conjugated to bovine serum albumin
Usage: Applications include ELISA (1/1,000-1/5,000); immunocytochemistry, immunohistochemistry (1/1,000-1/5,000); immunoblotting (western blot 1/1,000-1/2,000).

Hydroxytryptamine (Serotonin) Rabbit Polyclonal (Cat. #AB-T03, 50 µl)

Immunogen: Synthetic 5-Hydroxytryptamine conjugated to bovine serum albumin
Usage: Applications include ELISA (1/1,000-1/5,000), immunocytochemistry, immunohistochemistry (1/1,000-1/5,000), and immunoblotting (western blot, 1/1,000-1/2,000).

Noradrenaline Rabbit Polyclonal (Cat. #AB-T06, 50 µl)

Immunogen: Synthetic noradrenaline conjugated to bovine serum albumin
Usage: Applications include ELISA (1/1,000-1/5,000), immunocytochemistry (paraffin), immunohistochemistry (frozen, 1/1,000-1/5,000), immunoblotting (western blot 1/1,000-1/2,000).

Pseudomonas aeruginosa Rat Polyclonal (Cat. #AB-T058, 50 µl)

Immunogen: Pseudomonas aeruginosa total protein
Usage: Applications include ELISA (1/1,000-1/5,000) and immunoblotting (western blot 1/1,000-1/2,000).

Quinolinic acid Rabbit Polyclonal (Cat. #AB-T095, 50 µl)

Immunogen: Synthetic quinolinic acid conjugated to bovine serum albumin
Usage: Applications include ELISA (1/2,000-1/5,000) and immunohistochemistry / immunocytochemistry.

Trans-hydroxyproline Rabbit Polyclonal (Cat. #AB-T044, 50 µl)

Immunogen: Synthetic Trans-Hydroxyproline conjugated to bovine serum albumin
Usage: Applications include ELISA (1/2,000-1/5,000) and immunohistochemistry / immunocytochemistry.

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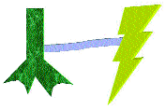

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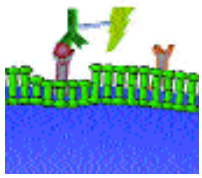
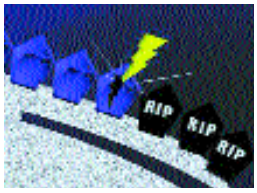


Cells that do not have the receptor will not be affected.

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.

GRAMOCILI

CEILS

STYENID

LAIZESUIV

MONCDOPU

How the scientist got the reaction he wanted

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

ANSWER: He provided the right . . .

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, 2009.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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Oct-Nov-Dec 2009
Volume 10, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Ablation of GRPR$^+$ Neurons in the Spinal Cord by Bombesin-Saporin Knocks Out Itch Sensation in Mice Without Affecting Pain Circuit

Contributed by Zhou-Feng Chen, Yang-Gang Sun, Zhong-Qiu Zhao, Xiu-Li Meng, Jun Yin, Xian-Yu Liu, Washington University Pain Center School of Medicine, Dept of Anesthesiology, St. Louis, MO 63141.

Unlike pain, itch sensation evokes scratching response instead of withdrawal behavior. However, itch and pain sensations share many similarities. Like chronic pain, chronic itch is a major clinical problem that affects the quality of life of millions of people and the underlying mechanisms are poorly understood. During the past decade, the concept that itch and pain are two distinct sensations has been increasingly appreciated and accepted. Nonetheless, itch-specific neurons are yet to be convincingly identified, raising the doubt about their very existence in the nervous system. In the spinal cord, spinothalamic tract (STT) neurons represent a key station for relaying both itch and pain sensations from the skin to the brain. Using electrophysiological recording, Andrew Craig's group found that a few mechanically insensitive STT neurons in cat spinal cord responded to histamine but not to mustard oil, a noxious chemical stimulus, and claimed that they represent a central pathway for itch.[1] However, these "histamine-selective neurons" were not examined for their responsiveness to other noxious chemical stimuli, such as capsaicin. Indeed, more recent studies in primates (Glenn Giesler and colleagues) found the itch-responsive STT neurons recorded were all responsive to capsaicin.[2] Therefore, whether there is an itch-specific circuit in the spinal cord remains unsettled.

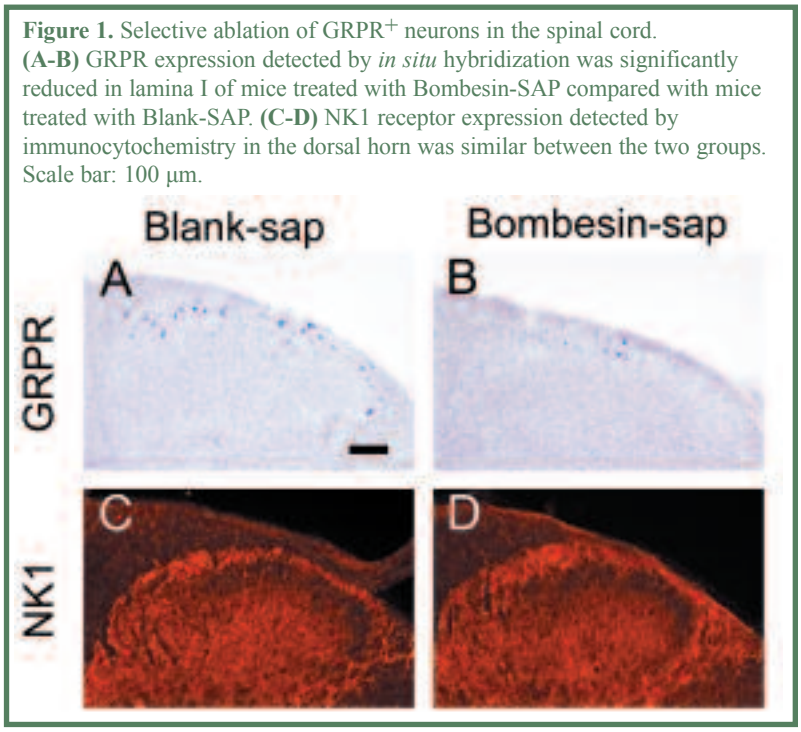

Figure 1. Selective ablation of GRPR$^+$ neurons in the spinal cord. **(A-B)** GRPR expression detected by *in situ* hybridization was significantly reduced in lamina I of mice treated with Bombesin-SAP compared with mice treated with Blank-SAP. **(C-D)** NK1 receptor expression detected by immunocytochemistry in the dorsal horn was similar between the two groups. Scale bar: 100 μm.

We previously found that gastrin-releasing peptide receptor (GRPR) is specifically expressed in lamina I of the spinal cord.[3] GRPR is a mammalian homolog of bombesin receptor, and a G-protein coupled receptor. Bombesin is a 14-amino acid peptide that was originally isolated from the skin of a frog, and gastrin-releasing peptide (GRP) is a mammalian homolog of bombesin. We found that mice lacking GRPR showed a significant reduction of scratching behavior to intradermal injection of a variety of pruritogenic (itchy) substances

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Inside this issue:

Newsletter Highlights

- 2009 SfN Posters *(page 2)*
- Anti-Melanopsin Protocol Correction *(page 5)*
- Teaser Winners *(page 5)*
- New Products: Leptin-SAP, Oxytocin-SAP, Anti-Basigin *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

2009 Society for Neuroscience Meeting - Booth 619

This year's Society for Neuroscience meeting is being held in Chicago, Illinois. ATS is located at **Booth 619** and we'll be there Saturday, October 17 through Wednesday, October 21. We hope you'll stop by and see us. Be sure to check out all the posters that feature targeted toxins. There are 37 this year. We do our best to find all of them, but please let us know if yours was left out. It is very helpful for abstract and journal articles to include the word 'saporin' somewhere in the abstract or text.

We have a listing posted on our website at: www.ATSbio.com/SfN2009posters

You can also pick up an itinerary of poster presentations at our booth. As always, we will be visiting the posters to select this years Poster of the Year award winner. The next issue of Targeting Trends will feature the winner's research using one or more targeted toxins. The SfN meeting is a great opportunity to interact with other molecular neurosurgeons to discuss your protocols and ideas, and to see what innovative work is being done throughout the world.

Make sure when you visit the booth to also ask for the 2010 calendar and our new 'green' tote bag. See you in Chicago!

Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

An anti-CD103 immunotoxin promotes long-term survival of pancreatic islet allografts

Zhang L, Moffatt-Bruce SD, Gaughan AA, Wang JJ, Rajab A, Hadley GA
Am J Transplant 9(9):2012-2023, 2009.

The integrin CD103 is suspected of promoting organ allograft rejection and graft-versus-host disease. A custom conjugation was done between the non-depleting CD103 antibody M290 and saporin. The conjugate was administered at 2.0 mg/kg to mice as an intraperitoneal injection (mouse IgG-SAP, Cat. #IT-18, was used as a control). The mice had previously received an islet transplant into a kidney capsule. Mice treated with M290-SAP were effectively depleted of CD103+ cells and had long-term acceptance of the allografts.

Medullary circuitry regulating rapid eye movement sleep and motor atonia

Vetrivelan R, Fuller PM, Tong Q, Lu J
J Neurosci 29(29):9361-9369, 2009.

Data concerning rapid-eye movement (REM) motor atonia in rats have not agreed with results seen in the large amount of data from cats. Here the authors traced the medullary networks in rats involved with the REM function. 120-300-ng injections of orexin-SAP (Cat. #IT-20) were administered to six different sites in the medulla. Ablation of orexin receptor-expressing neurons in one site in the ventromedial medulla resulted in intermittent loss of muscle atonia, indicating that glutaminergic neurons in this area are key components of the REM atonia circuit.

Please visit www.ATSbio.com to see a complete list of references.

The Neurokinin-1 Receptor Modulates the Methamphetamine-Induced Striatal Apoptosis and Nitric Oxide Formation in Mice

Zhu J, Xu W, Wang J, Ali SF, Angulo JA
J Neurochem [Epub Aug 13], 2009.

This study examined the role of neurokinin-1 receptors (NK-1r) on the methamphetamine-induced apoptosis of striatal neurons. 4 ng of SSP-SAP (Cat. #IT-11) or the control, saporin (Cat. #PR-01), was administered to the striatum of mice. Ablation of NK-1r-expressing striatal neurons resulted in a significant reduction of methamphetamine-induced apoptosis. The data suggest that the NK-1r circuitry in the striatum may be a target for treatment of methamphetamine abuse.

(continued on page 3)

Targeting Topics: Recent Scientific References

(continued from page 2)

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 [Epub Jun 24], 2009.

Selective ablation of rostral ventromedial (RVM) neurons expressing mu opioid receptors has been suggested as a treatment for pathological pain. This work investigated the side effects of a 0.5-µg injection of dermorphin-SAP (Cat. #IT-12) into the RVM. Saporin (Cat. #PR-01) was used as a control. Lesioned animals experienced a temporary increase in heart rate and systolic blood pressure, and mild microglial responses, but even these soon returned to normal. The data suggest this system has potential as a target for pain therapeutics.

Septal grafts restore cognitive abilities and amyloid precursor protein metabolism

Aztiria E, Cataudella T, Spampinato S, Leanza G
Neurobiol Aging 30(10):1614-1625, 2009.

It is suspected that there is a connection between the loss of cortical cholinergic input and the presence of β-amyloid precursor protein (APP) in Alzheimer's disease. After injecting 5 µg of 192-IgG-SAP (Cat. #IT-01) into the lateral ventricles of rats, the animals were given cholinergic-rich septal tissue grafts. The animals that received the grafts were able to restore APP levels to normal or near-normal, indicating that this type of therapy could at least slow cognitive dysfunction due to the lesion.

NGF is essential for hippocampal plasticity and learning

Conner JM, Franks KM, Titterness AK, Russell K, Merrill DA, Christie BR, Sejnowski TJ, Tuszynski MH
J Neurosci 29(35):10883-10889, 2009.

This work aimed to define NGF modulation of plasticity and function in adults. Rats received 50-ng injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum. Lesioned animals exhibited impaired retention of spatial memory and significantly reduced hippocampal long-term potentiation. These results indicate that NGF modulates neuronal plasticity and behavior by exerting effects on cholinergic projections to hippocampal and cortical targets.

Effects of chronic donepezil treatment and cholinergic deafferentation on parietal pyramidal neuron morphology

De Bartolo P, Gelfo F, Mandolesi L, Foti F, Cutuli D, Petrosini L
J Alzheimers Dis 17(1):177-191, 2009.

Donepezil has been shown to enhance cognitive functioning in both healthy patients and those suffering from dementia. This study examined whether donepezil treatment changes neocortical morphology in healthy or diseased brains. Rats received 4-µg bilateral injections of 192-IgG-SAP (Cat. #IT-01) into the lateral ventricles. Various morphological parameters were analyzed demonstrating that in the absence of cholinergic neurons donepezil prevented the compensatory response rather than enhanced function.

A cholinergic-dependent role for the entorhinal cortex in trace fear conditioning

Esclassan F, Coutureau E, Di Scala G, Marchand AR
J Neurosci 29(25):8087-8093, 2009.

Higher cognitive involvement can be modeled through the use of trace conditioning in simple associative tasks. Rats received several 20-80-ng injections of 192-IgG-SAP (Cat. #IT-01) into the entorhinal cortex (EC) in order to clarify the mechanisms that allow learning through the association of events that occur at different times. Cholinergic depletion of the EC did not result in a training deficit, indicating that these cells are not necessary for trace conditioning.

Neuroprotective effect of testosterone treatment on motoneuron recruitment following the death of nearby motoneurons

Fargo KN, Foster AM, Sengelaub DR
Dev Neurobiol 69(12):825-835, 2009.

Previous work has demonstrated that testosterone treatment can prevent dendritic atrophy due to death of nearby motoneurons. This experiment examined whether this protection extends to motor activation. Rats received a 1-µg injection of CTB-SAP (Cat. #IT-14) into each of the right bulbocavernosus and levator ani muscles. Animals treated with testosterone preserved more of the activity duration than untreated animals, as well as no decrease in motoneuron recruitment.

Effect of voluntary running on adult hippocampal neurogenesis in cholinergic lesioned mice

Ho NF, Han SP, Dawe GS
BMC Neurosci 10:57, 2009.

The act of running can induce hippocampal neurogenesis. In this work the authors investigated whether running can offset the loss of septohippocamal cholinergic neurons caused by a lesion using mu p75-SAP (Cat. #IT-16). Mice received 3.6 µg of the toxin into each lateral ventricle. Although the number of surviving neurons was similar in both lesioned and control animals, most of the progenitor cells in the lesioned animals could not survive without cholinergic input.

Hypocretin-2 saporin lesions of the ventrolateral periaquaductal gray (vlPAG) increase REM sleep in hypocretin knockout mice

Kaur S, Thankachan S, Begum S, Liu M, Blanco-Centurion C, Shiromani PJ
PLoS One 4(7):e6346, 2009.

Not all connections between narcolepsy and orexin are understood, since orexin neurons are located in the lateral hypothalamus and some sleep functions are controlled by the

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

brainstem. This experiment used 16.5-ng injections of orexin-SAP (Cat. #IT-20) into each side of the ventrolateral periaqueductal gray (v/PAG) to examine these connections. The results indicate that loss of orexin receptor-positive neurons in the v/PAG results in loss of inhibitory control over REM sleep, but does not cause cataplexy.

Role of layer 6 of V2 visual cortex in object-recognition memory

Lopez-Aranda MF, Lopez-Tellez JF, Navarro-Lobato I, Masmudi-Martin M, Gutierrez A, Khan ZU
Science 325(5936):87-89, 2009.

The authors examined the role of the V2 visual cortex in visual memory. Working with the prediction that object-recognition memory (ORM) control is centered in the V2 visual cortex, rats received 0.9-µg injections of OX7-SAP (Cat. #IT-02) into this area. Treatment with OX7-SAP eliminated virtually all neurons in layer 6 of area V2 of the visual cortex without damaging the hippocampus. The results indicate that this area of the visual cortex is important for ORM formation, but not storage.

Ketamine-induced deficit of auditory gating in the hippocampus of rats is alleviated by medial septal inactivation and antipsychotic drugs

Ma J, Tai SK, Leung LS
Psychopharmacol (Berl) [Epub Aug 5], 2009.

Schizophrenic patients do not experience the usual diminished response to repeated stimuli, otherwise known as gating. Gating loss can be caused by the administration of some psychotomimetic drugs. This study used 170-ng injections of 192-IgG-SAP (Cat. #IT-01) to examine the effect of ketamine on sensory gating loss. Elimination of septohippocampal cholinergic neurons alleviated the disruption of auditory gating caused by ketamine.

Immunotoxic depletion of microglia in mouse hippocampal slice cultures enhances ischemia-like neurodegeneration

Montero M, Gonzalez B, Zimmer J
Brain Res 1291:140-152, 2009.

Data has shown microglia to be both neuroprotective and neurodegenerative in cerebral ischemia. This study presents a method for removing microglia from hippocampal slice cultures. Hippocampal slices from mouse were incubated with 13-nM Mac-1-SAP (Cat. #IT-06) for 3 to 7 days. The slices were then exposed to oxygen-glucose deprivation. Those cultures lacking microglia displayed significantly higher degeneration of CA1 pyramidal cells, indicating a neuroprotective role for microglia in this model.

T-cell reconstitution without T-cell immunopathology in two models of T-cell-mediated tissue destruction

Penaloza-MacMaster P, Masopust D, Ahmed R
Immunology 128(2):164-171, 2009.

Although antigen-specific T-cells are vital to adaptive immune responses, they also contribute to a variety of diseases. In this work the authors examined the possibility of selectively removing epitope-specific T cells while preserving immune function. Biotinylated MHC class I monomers were combined with streptavidin-ZAP (Cat. #IT-27) and used in a mouse transferable T-cell-dependent neurological disease model. This technique resulted in a dramatic reduction in targeted antigen-specific T-cells with no observable bystander toxicity.

Cellular Basis of Itch Sensation

Sun YG, Zhao ZQ, Meng XL, Yin J, Liu XY, Chen ZF
Science [Epub Aug 6], 2009.

Whether itch and pain use separate neuronal pathways has long been a subject of debate. The authors injected 400 ng of bombesin-SAP (Cat. #IT-40) into the intrathecal space of mice and examined itch and pain behavior. Lesioned mice had dramatic deficits in all itch behavior tested regardless of the histamine-dependence of the itch. All pain behaviors, however, were left intact. These data indicate that the gastrin-releasing peptide receptor-expressing neurons are essential for itch transmission. *(See Cover Article.)*

Effects of the selective lesions of cholinergic septohippocampal neurons on different forms of memory and learning process

Dashniani MG, Beseliia GV, Maglakelidze GA, Burdzhanadze MA, Chkhikvishvili N
Georgian Med News 166):81-85, 2009.

The hippocampus is crucial for the ability to recollect everyday events and factual knowledge. Here the authors looked at the role of the septo-hippocampal cholinergic system of the medial septum in learning and memory. Rats received 200-ng injections of 192-IgG-SAP (Cat. #IT-01) into the medial septum. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The data suggest that although the septo-hippocampal region is essential for spatial learning, hippocampal acetylcholinesterase may not be essential for all types of hippocampal-dependent memory.

Neonatal stress affects vulnerability of cholinergic neurons and cognition in the rat: Involvement of the HPA axis

Aisa B, Gil-Bea FJ, Marcos B, Tordera R, Lasheras B, Del Rio J, Ramirez MJ
Psychoneuroendocrinol [Epub Jun 6], 2009.

Early adverse life events such as maternal separation (MS) can increase vulnerability to psychopathology as an adult. The authors administered bilateral intracerebroventricular 1-µg injections of 192-IgG-SAP (Cat. #IT-01) to MS rats, then analyzed choline acetyltransferase and acetylcholinesterase activity. Lesioned animals displayed reduced activity of both of these enzymes, as well as decreased glucocorticoid receptor density. The results suggest that vulnerability of basal forebrain cholinergic cells may be affected by the hypothalamic-pituitary-adrenal axis.

Please visit www.ATSbio.com to see a complete list of references.

Targeting Talk: Product Questions

by Dr. Douglas Lappi

CORRECTION: The protocol printed in Targeting Trends Volume 10, Issue 3 was incorrect. The corrected protocol is presented at right.

Q: We're interested in trying out your melanopsin antibody (Cat. #AB-N38) using immunohistochemistry in mouse retina. Do you have a recommended protocol?

A: Please see corrected protocol from Panda *et al*. This protocol is also available on our website. Just click on "Protocols" on our Home Page. ***www.ATSbio.com***

Panda S. *et al*. 2002. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science* 298(5601):2213-2216.

Targeting Teaser Winners

The solution to the puzzle was:

Jumbles: MICROGLIA
SLICE
DENSITY
VISUALIZE
COMPOUND

Answer: He provided the right . . .STIMULUS

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

WINNERS: David Looney, UCSD Pathology, La Jolla, CA * Megan Green, Univ TX Health Science Ctr Pharmacology, San Antonio, TX * Erica Edwards, Oklahoma Medical Research Fdtn, Oklahoma City, OK * Shikha Gaur, City of Hope Clinical & Molecular Pharmacology, Duarte, CA * Bruce Pappas, Carleton Univ, Ottawa, ON * Ruth Stornetta, Univ Virginia Pharmacology, Charlottesville, VA * April Price, UCSF Medicine, San Francisco, CA * Brigitta Peteri, CNRS FRE 3094, Nice, FRANCE * Glenn Kageyama, Cal Poly Pomona Univ, Pomona, CA * Jean Peduzzi, Wayne State Univ School of Medicine, Detroit, MI * Muthu Kumara gnana sammandh, National Univ Singapore, Bioengineering, Singapore * Seto Chice, SUNY-HSC, Brooklyn, NY * Cai Peng, ApoPharma Inc, Ottawa, ON * Elia Nahas, McGill Univ, Montreal, QC * Ashley Linder, UCSD Neurobiology, La Jolla, CA

Solve the Teaser online at:
http://www.atsbio.com/news/09q4_teaser.html

Anti-Melanopsin Immunostaining Protocol
*** corrected ***

Remove the corneas, and postfix eyes at 4°C for 24 hours in 4% paraformaldehyde in phosphate-buffered saline (PBS). Remove lenses.

Cryoprotect eyecups for sectioning at 4°C for 24 hours in 30% sucrose in PBS; embed the eyecups in OCT medium (Sakura Finetek, Torrance, CA), freeze, section (16-20 µm), and thaw-mount onto gelatin-coated slides.

Dissect retinas destined for flat-mounting from eyecups immediately after postfixation, stretch onto filter paper, and process in 1.5-ml microfuge tubes.

Wash tissue (slides and flat-mounts) 3 times (10 min, 4°C) in Tris-buffered saline (TBS, Quality Biological, Gaithersburg, MD) and block for 30 min at 4°C in 1.5% normal goat serum in TBS.

Incubate tissue for 24 hr at 4°C in a 1:2,500 dilution of anti-Melanopsin UF006 (Cat. #AB-N38) in a TBS-incubating buffer containing 1% bovine serum albumin, 0.25% carrageenan lambda and 0.003% Triton X-100.

Wash slides and flat mounts three times in TBS (10 min, 4°C) and incubate for 1 hour at 22°C in Cy3-conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:500 in TBS incubating buffer.

Wash 3 final times in TBS (10 min, 22°C).

Remove flat-mounts from the filter paper and transfer onto glass microscope slides. Mount flat-mounts and sections in DAPI-containing Vectashield (Vector Laboratories, Burlingame, CA), coverslip, and seal with clear fingernail polish.

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American Association for Cancer Research
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Ablation of GRPR+ Neurons By Bombesin-SAP Knocks Out Itch Sensation

(continued from page 1)
compared with their littermate mice.[3] In contrast, these mutant mice showed normal pain behaviors. Our results suggested that GRPR is important for itch but not for pain sensation in the spinal cord.

Our work on GRPR raised an outstanding question: Are GRPR+ neurons dedicated to itch sensation? The present study was designed to address this question. We took advantage of the fact that bombesin can bind GRPR with a high affinity and become internalized upon binding, and ablated GRPR+ neurons by injecting Bombesin-SAP (Cat. #IT-40) into the spinal cord of mice.[4] After two weeks, we found that up to 80% of GRPR+ neurons were selectively ablated (Fig. 1). Interestingly, injection of bombesin-saporin did not affect expression of NK1 receptor that is expressed in the majority of STT neurons (Fig. 1). Strikingly, mice lacking GRPR+ neurons in the spinal cord showed profound deficits in their scratching behavior in response to several pruritogens, including both histamine-dependent (e.g. serotonin) and histamine-independent ones (e.g. chloroquine). Some mice were unable to scratch at all, no matter how potent the pruritogen (Fig. 2). The scratching behavior of mice treated with Bombesin-SAP was also nearly abolished when an immunotherapy agent called DCP that can cause intense itching in people was used to induce long-lasting scratching (Fig. 2).

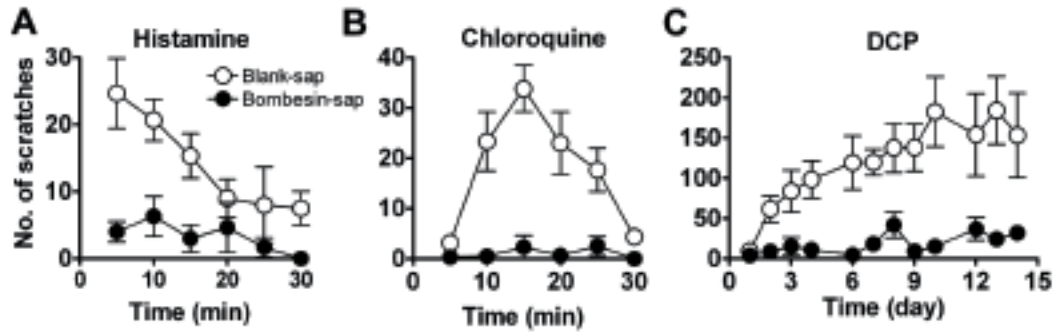


Figure 2. Selective ablation of GRPR+ neurons nearly abolished scratching behaviors. **(A)** Histamine-evoked scratching behavior in mice treated with Bombesin-SAP was almost lost compared with the Blank-SAP control (500 μg/50 μl, $P < 0.001$). **(B)** Chloroquine-evoked scratching behavior in Bombesin-SAP-treated mice was also absent (200 μg/50 μl) ($P < 0.001$). **(C)** Scratching behavior evoked by diphenylcyclopropenone (DCP) was nearly blocked in mice treated with Bombesin-SAP compared with mice treated with Blank-SAP ($P < 0.001$). Two-way repeated measured analysis of variance (ANOVA). n = 6~9 for each group. Data with error bars represent mean ± SEM.

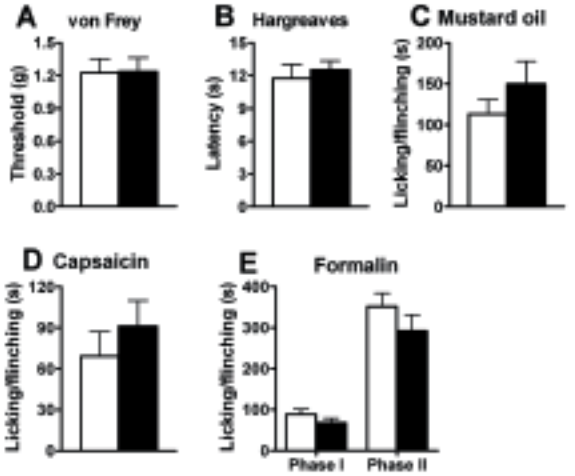


Figure 3. Normal pain behaviors in mice treated with Bombesin-SAP. (A) Mechanical sensitivity in Bombesin-SAP-treated mice as measured by paw withdrawal threshold upon exposure to von Frey filaments was comparable to mice treated with Blank-SAP (Cat. #IT-21). $P > 0.05$. (B) Responses to noxious thermal stimulation measured by the paw withdrawal latency (Hargreaves test) were indistin-guishable between groups. $P > 0.05$. (C) Spontaneous pain response in mustard oil test was comparable between the two groups. $P > 0.05$. (D) Spontaneous pain response induced by intraplantar injection of capsaicin (0.1%) was comparable between groups. $P > 0.05$. (E) Spontaneous pain responses in first (0~10 min) and second phase (10~60 min) of the formalin test was comparable between mice treated with Blank-SAP and Bombesin-SAP.
$P > 0.05$.Student's t-test. n = 6~9 for each group. Black bars: the Bombesin-SAP group; white bars: the Blank-SAP group. Data with error bars represent mean ± SEM.

The scratching deficits of these mice treated with Bombesin-SAP are much more comlete than that of GRPR mutant mice which showed modest decreased response compared with wild-type mice to histamine or histamine-dependent pruriotogens. The lack of scratching responses is unlikely due to motor deficit because these mice showed normal motor function. The most unexpected results are that mice treated with Bombesin-SAP showed normal pain behaviors in response to different types of painful stimuli, including thermal, mechanical and chemical stimuli (Fig. 3). For noxious chemical stimuli, we used formalin, mustard oil, capsaicin and complete Freund adjuvant.[4] Our studies suggest that GRPR+ neurons are itch-specific. Given that NK1 expression is normal in mice treated with Bombesin-SAP and STT neurons are critical for pain sensation, these results suggest that GRPR+ neurons and STT neurons are two non-overlapping subpopulations in lamina I of the spinal cord. Together, we provide the most comprehensive behavioral evidence supporting the idea that there is a neural circuit hard-wired specifically to relay itch sensation in the spinal cord. Identification of itch circuit in mice has important therapeutic implications, because it is now possible to design novel anti-pruritus treatments based on the blockage of the itch pathway without compromising other somatic sensations.

References:

1. Andrew D, Craig AD (2001) Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. *Nat Neurosci* 4:72-77.
2. Davidson S, Zhang X, Yoon CH, Khasabov SG, Simone DA, Giesler GJ Jr (2007) The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. *J Neurosci* 27(37):10007-10014.
3. Sun YG, Chen ZF (2007) A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. *Nature* 448(7154):700-703.
4. Sun YG, Zhao ZQ, Meng XL, Yin J, Liu XY, Chen ZF (2009), Cellular Basis of Itch Sensation. *Science*, 325(5947):1531-1534.

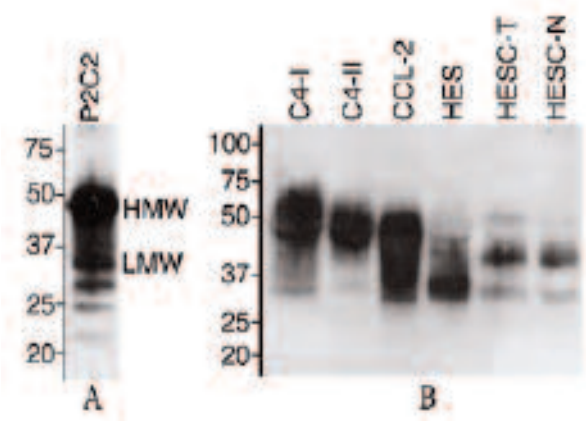
Targeting Tools: Featured Products

Leptin-SAP

Leptin-SAP (Cat. #IT-47) is a conjugate (molecular weight 70 kDa) 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.

Oxytocin-SAP

Oxytocin-SAP (Cat. #IT-46) is a conjugate (molecular weight 31 kDa) between the native oxytocin peptide and saporin. Oxytocin is a nine amino acid peptide with a structure similar to vasopressin, and is a hormone released by the posterior pituitary. Peripheral activity of oxytocin is linked primarily to lactation and labor-related uterine contractions. Within the brain, oxytocin receptor-positive neurons have been studied with regard to their sensitivity to leptin and involvement in satiety.



A: Anti-basigin recognizes native glycosylated basigin in HeLa cell lysate. B: The basigin monoclonal antibody immunoprecipitates glycosylated basigin proteins from a variety of human cell lines such as cervical carcinomas (C4-I/C4-II), uterine epithelials (HES), and uterine fibroblasts (HESC).

Anti-Basigin

This mouse monoclonal antibody (Cat. #AB-42; Clone P2C2) recognizes human Basigin. The antibody is purified by ammonium sulfate precipitation on conditioned medium and then further purified through Protein-A. The antibody is routinely tested by flow cytometry. The immunoglobulin superfamily protein basigin (EMMPRIN/CD147) is a cell surface glycoprotein expressed by tumor cells that stimulates matrix metalloproteinase (MMP) and vascular endothelial growth factor (VEGF). With basigin's ability to stimulate the expression of molecules that participate in tissue remodeling and angiogenesis, it may prove to be a potential target for the development of methods to inhibit metastasis.

Bombesin-SAP

Bombesin-SAP (Cat. #IT-40) specifically targets and eliminates gastrin-releasing peptide receptor (GRPR)-positive cells. This targeted toxin (molecular weight 31.8 kDa) is a chemical conjugate of bombesin and the ribosome-inactivating protein, saporin. Please see cover article (pp. 1, 6) for an example of its experimental use.

Gangsta takes one small step for all cat-kind.

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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.

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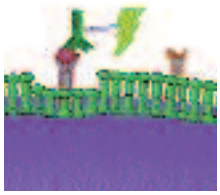
§ or anything recognized on the cell surface and internalized.

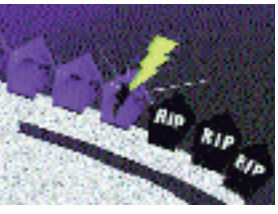
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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.

CHIT
□□□□

MASTHINIE
□□□□□○□□○

HACCRTS
○□□□□□○

NATTUM
○□□□□○

STENNIE
□□○□□□□

What the scientist thought about his plants when he left the lab each evening.

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

ANSWER: He . . . "□□□□" □□□□

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, 2009.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 5.

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