October 2000
Volume 1, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- Toxicology/Safety studies with SP-SAP to begin
- Mouse p75 immunotoxin to be released at 2000 SFN Meeting
- Second immunotoxins: use your monoclonal or polyclonal to target cells

ADVANCED TARGETING SYSTEMS

CBF Lesioning in Rabbits

contributed by Dr. Thomas Beach

Sun Health Research Institute, Sun City, AZ

Our hypothesis, developed through our own human and animal studies[1-6] and the cell culture work of others (beginning with Nitsch[7]), is that the normal, age-related loss of cortical cholinergic innervation leads to Aβ deposition and Alzheimer's disease. To test this hypothesis, we have been using a saporin-conjugated antibody to lesion the cholinergic basal forebrain (CBF) of rabbits. The antibody is the ME20.4 monoclonal[8] against the low affinity nerve growth factor receptor, also known as the p75 neurotrophin receptor (p75NTR). This approach had already been shown to result in an effective and specific lesion in rats, but we wished to use an animal with an Aβ sequence identical to that of humans (the rat sequence differs by 3 amino acids), since we considered that, because of this difference, rats may be less likely to produce Aβ deposits. As the IgG192 anti-p75NTR antibody used for rats does not recognize rabbit CBF neurons, we tested the ME20.4 antibody

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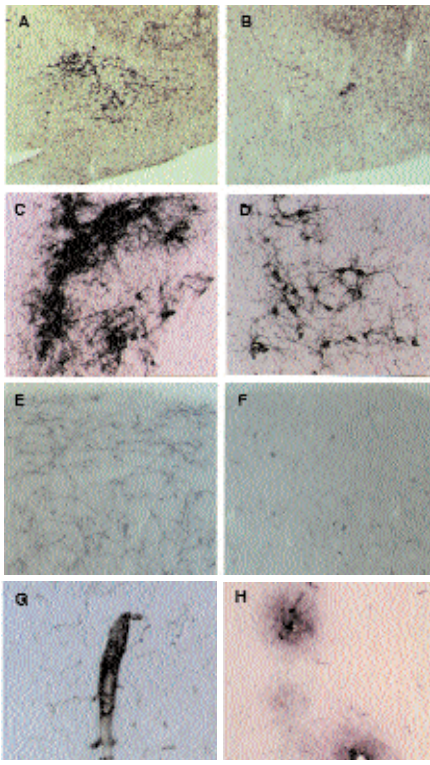

Fig 1 Histologic demonstration of the nbm lesion and resultant Aβ deposition. A,B: rabbit nbm area in control (A) and immunotoxin-treated animals (B), stained for AChE. Note the marked depletion of nbm neurons in the latter. C,D: rabbit nbm in control (C) and treated (D) animal, stained immunohistochemically for p75 NTR. Note the depletion of neurons in the treated animal. E,F: frontal cortex from control (E) and treated (F) animals, stained for AChE. Note eradication of cholinergic fibers in the latter. G,H: Aβ deposition in cerebral cortex of lesioned animals. Aβ is deposited in blood vessel walls (G) and in the perivascular neuropil (H).

Chronic Pain Therapeutic

In April of this year ATS applied for funding from the National Institute for Mental Health (NIMH) for the toxicology studies of Substance P-Saporin (SP-SAP). This $674,000 supplement to a previous NIMH Small Business Innovative Research grant will cover one of the FDA-required safety and toxicology studies and will bring us closer to clinical trials in humans. On August 14, ATS received notice of the reviewers' score of these studies. ATS is pleased with the reviewers' assessment and is optimistic about funding. Collaborating with ATS on this grant are two scientists who are experts in their fields: Dr. Tony Yaksh (UCSD, Dept. of Anesthesiology) who will be heading the toxicology/safety experiments in animals, and Dr. Patrick Mantyh (Univ. Minnesota), who will provide analysis and

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NIDA funds ATS research on Galanin

Advanced Targeting Systems (ATS) has just been awarded a $100,000 research grant from the National Institute on Drug Abuse (NIDA). This Phase I SBIR (Small Business Innovation Research) grant proposes to develop a research reagent to study the function of the neuropeptide, galanin. This will be an important tool for scientists to use in the study of galanin's influence in many biological systems: pain, depression, anxiety, memory and feeding.

Galanin is a 29/30 amino acid peptide and asserts its biological effect through G-protein-coupled receptors that are widely distributed. In the brain, I^{125}-galanin or galanin-analog binding studies show high levels of binding in the cerebral cortex, thalamus, pons, and cerebellum. Outside the brain, the anterior pituitary, pituitary tumors, astrocytes, spinal cord, and gastric and jejunal smooth muscle show ligand binding (1).

At the molecular level, multiple second messenger pathways are activated: inhibition of cyclic AMP, activation of channels including ATP-sensitive K+ channels, inhibition of L-type and N-type calcium channels, stimulation of inositol phospholipid turnover, stimulation and inhibition of calcium mobilization, stimulation of phospholipase A2, activation of MAP kinase, mitogenesis and stimulation of cyclic AMP accumulation (1).

This diversity of biochemical activities leads to a wide array of effects in biological systems. Galanin is reported to modulate feeding and sexual behavior, play a role in pain transmission, affect depression, and may be involved in the pathogenesis of Alzheimer's disease (2).

For the next six months, ATS will develop and test a galanin-saporin fusion protein to be offered commercially to research scientists worldwide. This targeted toxin will be a valuable research tool to study biological systems and for the study of drug abuse. The presence of galanin receptor-expressing cells in systems that control depression, pain and eating disorders, along with the connection between the opiate receptors, may indicate that galanin plays a role in drug abuse. The removal of these neurons, and their effects on neurons that express opiate receptors, will be a powerful tool to examine the role of galanin in drug use and abuse.

1. Williams RL; Hilton DJ; Pease S; Willson TA; Stewart CL; Gearing DP; Wagner EF; Metcalf D; Nicola NA; Gough NM. *Nature* 1988, 336, 684-687.
2. Kask K; Berthold M; Bartfai T. *Life Sci*. 1997, 60 (18), 1523-1533.

Dr. Douglas Lappi is Principal Investigator on the recently-funded SBIR Phase I grant from NIDA.

Upcoming Events

Society for Neuroscience
New Orleans, LA • November 4-9, 2000
Booth #438

Chronic Pain Therapeutic

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imaging of tissue samples from the study. Dr. Douglas Lappi (President and Senior Scientist at ATS) is Principal Investigator for this project and plans to begin synthesis of SP-SAP for use in these studies early in October.

The toxicology/safety studies will provide important information about the way SP-SAP may be used to treat intractable pain in humans. First, these experiments should tell us whether SP-SAP has toxic side effects. Second, they should give indications of how safe the drug will be at various doses. Finally, this will be the first time we will study the way the body reacts to the drug: how quickly it is absorbed, how it is broken down in the body, whether any of these breakdown products are toxic, and how quickly the drug and its breakdown products are eliminated from the body. If, after this intensive series of experiments, SP-SAP is determined to be safe to use in humans, ATS will initiate the first clinical trials.

The FDA has advised us that SP-SAP may best be developed as an orphan drug for treatment of pain in patients with terminal cancer. ATS will interact closely with the FDA during the drug development process and will publish updates in our quarterly newsletter and on our website at ATSbio.com.

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Impaired acquisition of a Morris water maze task following selective destruction of cerebellar Purkinje cells with OX7-saporin.

Gandhi CC, Kelly RM, Wiley RG, Walsh TJ.
Behav Brain Res 109(1):37-47, 2000.

The cerebellum has been associated with the control of motor activity and voluntary movements. Recent data have shown the cerebellum may also play a role in "higher order" processes such as learning, language, and cognition. Using 2 μg OX7-SAP (Catalog #IT-02) by i.c.v.. injection, Gandhi *et al*. selectively eliminated Purkinje cells in rat cerebellum in order to examine the ability of treated animals to complete a water maze task. Elimination of these cells significantly impaired the ability of the rats to complete the task, suggesting the cerebellum is involved in learning.

Ectopic noradrenergic hyperinnervation does not functionally compensate for neonatal forebrain acetylcholine lesion.

Pappas BA, Nguyen T, Brownlee B, Tanasoiu D, Fortin T, Sherren N.
Brain Res 867(1-2):90-99, 2000.

Removal of cholinergic forebrain neurons in the neonatal rat causes an ingrowth of hippocampal material to the affected area. The behavioral effect of this treatment increases working, but not reference memory errors on the radial arm maze. Pappas *et al*. used 300 ng 192-Saporin (Catalog #IT-01) by i.c.v. injection to lesion the forebrain of 1- to 3-day old rats coupled with a 6-OHDA lesion to remove hippocampal ingrowths in order to investigate whether these neurons can compensate for cholinergic function in memory. Their results indicate noradrenergic neurons from the hippocampus do not functionally compensate for loss of CBF neurons even though losses of these neurons did not drastically affect the behavior of these animals.

NGF-mediated alteration of NF-κB binding activity after partial immunolesions to rat cholinergic basal forebrain neurons.

Gu Z, Toliver-Kinsky T, Glasgow J, Werrbach-Perez K, Perez-Polo JR.
Intl J Dev Neurosci 18(4-5):455-468, 2000.

After injecting 1.3 μg 192-Saporin (Catalog #IT-01) into the lateral ventricle of rat brain, followed by infusion of NGF antibody, Gu *et al*. report changes in the activity of the transcription factor NF-κB. Aged rodent brains show an increase in NF-κB activity. This model creates a tool to investigate decreased cholinergic function that is often associated with memory loss and cognitive deficits in the elderly and particularly in patients with Alzheimer's disease.

Preferential destruction of cerebellar Purkinje cells by OX7-saporin.

Angner RT, Kelly RM, Wiley RG, Walsh TJ, Reuhl KR.
Neurotoxicology 21(3):395-403, 2000.

Purkinje cells function as inhibitors and are the sole output of the cerebellar cortex. Angner *et al*. eliminate these cells in rats with 1-2 μg OX7-SAP (Catalog #IT-02), an immunotoxin that binds the Thy 1.1 antigen. The treated rats show effects of loss of inhibitory control, including a time-dependent increase in motor activity and decreased motor coordination.

Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation.

Gill TM, Sarter M, Givens B.
J Neurosci 20(12):4745-4757, 2000.

Preliminary evidence suggests that demands on attention levels are associated with changes in levels of cortical acetylcholine. Gill *et al*. used .05 μg 192-Saporin (Catalog #IT-01) by intracortical infusion to demonstrate the role cholinergic neurons play in the ability of rats to pay attention. The researchers monitored medial prefrontal cortex (MPC) activity in the rat brain before and after elimination of cholinergic neurons with 192-Saporin. The results suggest that the cholinergic inputs to the MPC influence the increases of neuronal activity associated with paying attention.

Immunolocalization of the cocaine- and antidepressant-sensitive 1-norepinephrine transporter.

Schroeter S, Apparsundaram S, Wiley RG, Miner LH, Sesack SR, Blakely RD.
J Comp Neurol 420(2):211-232, 2000.

Norepinephrine transporters are involved in the response to multiple antidepressants and psychostimulants, but the expression of these proteins has not yet been characterized in the central nervous system. Schroeter *et al*. used an antibody to a cytoplasmic epitope of norepinephrine transporters to map the transporters to noradrenergic neuronal somata, axons, and dendrites. To verify the specificity of the antibody the researchers injected 10 μg of anti-DBH-SAP (Catalog #IT-03) in the left lateral ventricle of rats to destroy the noradrenergic neurons, confirming the specificity of the norepinephrine transporter antibody. Treatment with anti-DBH-SAP completely removed norepinephrine transporter immunoreactivity.

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Targeting Topics: Recent Scientific References

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Basal forebrain neurons suppress amygdala kindling via cortical but not hippocampal cholinergic projections in rat.

Ferencz I, Leanza G, Nasnobashvili A, Kokaia M, Lindvall O.
Eur J Neurosci 12(6):2107-2116, 2000.

Cholinergic mechanisms have been implicated in human epilepsy, possibly in the role of seizure suppression. Ferencz *et al*. used 2.5 µg 192-Saporin (Catalog #IT-01) by i.c.v. injection to investigate the effect of eliminating cholinergic projections to the hippocampal formation and cerebral cortex on the induction of epilepsy through electrical stimulation of the rat brain. The researchers used the specificity of 192-Saporin to determine that the loss of specific projections to the amygdala accelerates development of seizures. The hippocampus does not influence this process.

Repeated immunolesions display diminished stress response signal.

Gu Z, Yu J, Werrbach-Perez K, Perez-Polo JR.
Int J Dev Neurosci 18(2-3):177-183, 2000.

Cholinergic neurons in the basal forebrain are involved in neurotrophin release in general injury response although this response is impaired in the aged individual. Addition of pharmacological doses of NGF can repair this mechanism. Gu *et al*. used 192-Saporin (Catalog #IT-01) to model the endogenous stimulation of NGF in response to injury. They found that a one-time administration of 192-Saporin was more effective than chronic repeated administrations for inducing an increase in NGF levels. These results indicate that chronic lesions may cause a desensitization that differs from the acute toxic model.

Cholinergic excitation of septohippocampal GABA but not cholinergic neurons: implications for learning and memory.

Wu M, Shanabrough M, Leranth C, Alreja M.
J Neurosci 20(10):3900-3908, 2000.

It has long been assumed that the drug-induced enhancement of learning and memory in both young and aged rats was accomplished through a cholinergic pathway in the hippocampus. Wu *et al*. used a fluorescent labeling molecule, 192-IgG conjugated to Cy3 (Custom Service from ATS) to visualize these neurons. They found that the effects of cognition-enhancing drugs are not facilitated through action on cholinergic neurons. Instead, activation of GABA neurons is implicated in this model.

Attenuation of the bidirectional effects of chlordiazepoxide and FG 7142 on conditioned response suppression and associated cardiovascular reactivity by loss of cortical cholinergic inputs.

Stowell JR, Berntson GG, Sarter M.
Psychopharmacol 150(2):141-149, 2000.

The benzodiazepine receptor (BR) is involved in anxiety. It has been hypothesized that cholinergic projections from the CBF are necessary for modulation of the BR by agonists and inverse agonists. Stowell *et al*. directly injected 0.18 µg 192-Saporin (Cat #IT-01) into each hemisphere of the CBF in adult rats. The treated rats had altered responses to external stimuli during an operant conditioned task. These results indicate that the CBF plays an important role in response to fear and anxiety-related stimuli. This system may also mediate the actions of BR ligands.

Intracerebroventricular infusion of CHO5, a rat monoclonal antibody directed against mouse low-affinity nerve growth factor receptor (p75NTR), specifically labels basal forebrain cholinergic neurons in mouse brain.

Rossner, S, Schliebs, R, Bigl, V.
Metab Brain Dis 15(1):17-27, 2000.

192-Saporin (Catalog #IT-01) has long been a useful tool for neurobiological research in the rat. For various reasons, many researchers want to perform the same studies in the mouse but have been prevented from doing so by the lack of a suitable antibody against the mouse p75NTR. Rossner *et al*. describe a rat monoclonal antibody against the mouse p75NTR (Catalog #AB-N02) that demonstrates co-localization of p75NTR and ChAT, and also co-localization of p75NTR and TrkA in the mouse basal forebrain. Internalization and retrograde transport of this antibody in cholinergic basal forebrain neurons is also shown. This evidence indicates that the anti-mouse p75NTR will be effective for use as an immunotoxin.

(NOTE: ATS plans release of the mouse-specific p75 immunotoxin in early November -- see new product release notes on page 7 of this newsletter.)

Please visit our website (www.ATSbio.com) to see the extensive list of references for all of our products.

Targeting Talk: Second Immunotoxins

Q: What is a second immunotoxin?

A: ATS's second immunotoxins are conjugations of a secondary antibody (either goat anti-mouse IgG or goat anti-rabbit IgG) to the ribosome-inactivating protein, saporin.

Q: How does a second immunotoxin target?

A: The second immunotoxin uses the secondary antibody to "piggyback" onto your primary antibody in order to evaluate the ability of the primary antibody to internalize.

Q: What happens when the second immunotoxin gets inside the cell?

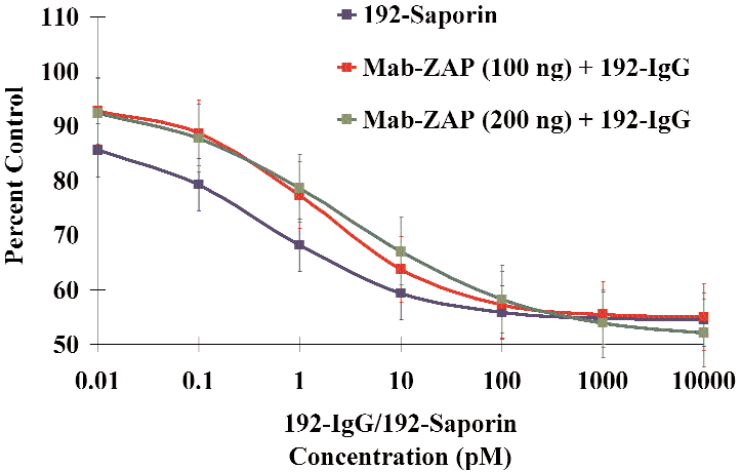
A: If the second immunotoxin is internalized, saporin will inactivate the ribosomes of the cell, thereby causing cell death.

Q: Are there different types of second immunotoxins available?

A: ATS has two products available. 1) Mab-ZAP (Catalog #IT-04) that recognizes mouse primary antibody, and 2) Rab-ZAP (Catalog #IT-05) that recognizes rabbit primary antibody.

Q: What is the ratio of antibody to second immunotoxin for in vitro testing?

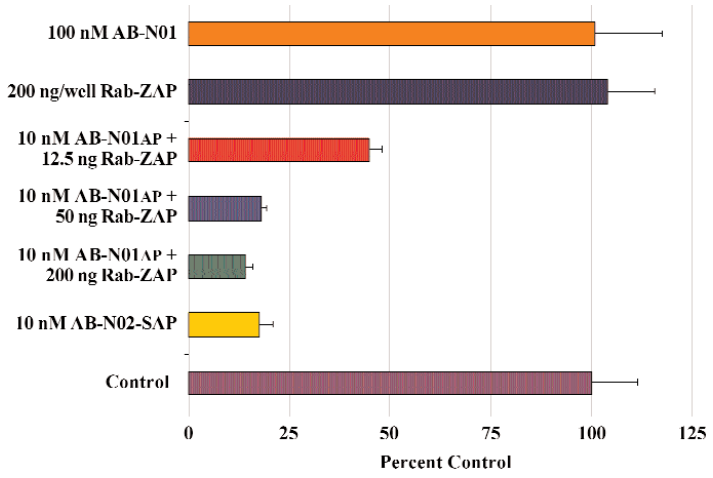
A: Both Mab-ZAP and Rab-ZAP have been shown effective in concentrations ranging from 0.5 to 2 moles of primary antibody per mole of second immunotoxin.



Mab-ZAP

Catalog #IT-04

uses your mouse primary antibody to target and eliminate cells



Rab-ZAP

Catalog #IT-05

uses your rabbit primary antibody to target and eliminate cells

Product Pricing

Mab-ZAP (IT-04) and Rab-ZAP (IT-05)

Individually 25 µg $165
100 µg $625

Kit, with unconjugated antibody and saporin
25 µg $200
100 µg $750

For more information, call ATS for a free reprint:

Kohls, MD and Lappi, DA. Mab-ZAP: A tool for evaluating antibody efficacy for use in an immunotoxin. *BioTechniques* 28(1):162-165, 2000.

CBF Lesioning in Rabbits

(continued from page 1)

and found, in preliminary immunohistochemical studies, that it stained the rabbit CBF neurons beautifully (Fig 1c,d). We obtained the ME20.4-producing cells from the American Type Culture Collection (ATCC) and forwarded these to Advanced Targeting Systems for antibody production and coupling to saporin.

We injected 30-40 μl of the conjugate into the lateral ventricles of New Zealand white rabbits and sacrificed the animals at 5 weeks, 3 months and 6 months. At all time intervals, we found that lesioned animals showed loss of CBF neurons (Fig 1a-d), cortical ChAT decrements of 50-70%, and profound loss of cholinergic nerve fibers (Fig 1e,f). All lesioned animals showed Aβ deposition in cerebral blood vessel walls and as perivascular diffuse deposits in the neuropil (Figure 1g,h). Biochemical (ELISA) measurements confirmed that Aβ concentrations were elevated in the cerebral cortex, 2.5-fold and 8-fold, respectively, for the forms of the peptide ending in 40 and 42. This model may recapitulate the events leading to Aβ deposition in aging and the sporadic form of Alzheimer's disease.

All lesioned animals showed Aβ deposition in cerebral blood vessel walls and as perivascular diffuse deposits in the neuropil.

References

1. Beach TG and McGeer EG. *Acta Neuropathol*. 83:292-299, 1992.
2. Beach TG, Walker, D.G., Cynader MS and Hughes LH. *Neurosci. Lett*. 142:253-256, 1996.
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4. Beach, TG, Sue, LI, Scott, S and Sparks, DL. *Alz. Reports* 1:375-380, 1998.
5. Beach, TG, Potter, PE, Kuo, Y-M, Emmerling, MR, Durham, RA, Webster, SD, Walker, DG, Sue, LI, Scott, S, Layne, KJ and Roher, AE. *Neurosci. Lett*. 283: 9-12, 2000.
6. Beach, TG, Kuo, Y-M, Spiegel, K, Emmerling, MR, Sue, LI, Kokjohn, K and Roher, AE. *J. Neuropathol. Exp. Neurol*. 59:308-313, 2000.
7. Nitsch, RM, Slack, BE, Wurtman, RJ, and Growdon, JH. *Science* 258:304-307, 1992.
8. Fine, A, Hoyle, C, Maclean, CJ, Levatte, TL, Baker, HF and Ridley, RM. *Neuroscience* 81:331-343, 1997.

ATS researcher, Matthew Kohls, works on new product development.

Targeting Ticklers

Actual Excerpts From Student Science Exam Papers:

1. The theory of evolution was greatly objected to because it made man think.
2. Algebraical symbols are used when you do not know what you're talking about.
3. A circle is a line which meets its other end without ending.
4. The pistol of a flower is its only protection against insects.
5. The moon is a planet just like the Earth, only it is even deader.
6. Blood flows down one leg and up the other.
7. It is a well-known fact that a deceased body harms the mind.
8. For drowning: climb on top of the person and move up and down to make artificial perspiration.
9. To remove dust from the eye, pull the eye down over the nose.
10. When you smell an odorless gas, it is probably carbon monoxide.

Targeting Tools: New Products

Mouse-Specific p75 Immunotoxin

The long-sought mouse cholinergic toxin has been found! The mouse cholinergic immunotoxin is being released for sale at the Society for Neuroscience meeting in New Orleans, November 4-9, 2000! For years ATS has received requests for a molecule that would eliminate cholinergic neurons in the basal forebrain of mice in a manner that 192-Saporin does in the rat. Because of the increasing importance of the mouse in understanding behavioral systems such as learning, memory and attention, there has been a constant interest in a molecule that could do the work. For instance, many transgenic models have given new insights into our understanding of Alzheimer's Disease (AD), but few of them offered a clear neurodegeneration of one of the most important symptoms: cholinergic denervation. In 1995, ATS, in collaboration with Dr. Joanne Berger-Sweeney of Wellesley College, began its search for the elusive mouse p75 cholinergic toxin. The expression of p75, the low affinity neurotrophin receptor, on the surface of cholinergic basal forebrain (CBF) neurons provided an opportunity to target these neurons in the mouse, just as we had done in the rat. Conjugation of an antibody that targets p75 to saporin has produced a cytotoxin that eliminates the CBF neurons while sparing neighboring neurons that express GAD, calbindin and parvalbumin. Differences between the mouse cholinergic toxin and the rat version, and methods to use the toxin can be discussed with the experts at the ATS booth at the Society for Neuroscience meeting. We also recommend visiting the poster at the Society for Neuroscience meeting that describes the properties and activities of the new immunotoxin: *A Specific Cholinergic Immunotoxin in Mice, J.E. Berger-Sweeney, S.L. Murg, M.G. Baxter, N.A. Stearns, D.A. Lappi, Abstract ID 4342, Nov 7, 2000 1:00 - 2:00 PM, Hall G-J.*

Coming Soon!

Denise Higgins, Director of Business Development, negotiates licensing agreements and coordinates the marketing of new products.

Neurotransmitter Antibodies

Polyclonals

Raised in rabbits

5-Hydroxytryptophan, AB-T09
Acetylcholine, AB-T02
Dopamine, AB-T07
GABA, AB-T10
Glutamate, AB-T08
Glutathione, AB-T01
Noradrenaline, AB-T06
Serotonin, AB-T03
Tryptamine, AB-T04
Tryptophan, AB-T05

Monoclonals

Raised in BALB/c mice

Dopamine, AB-T11
Glutamate, AB-T12

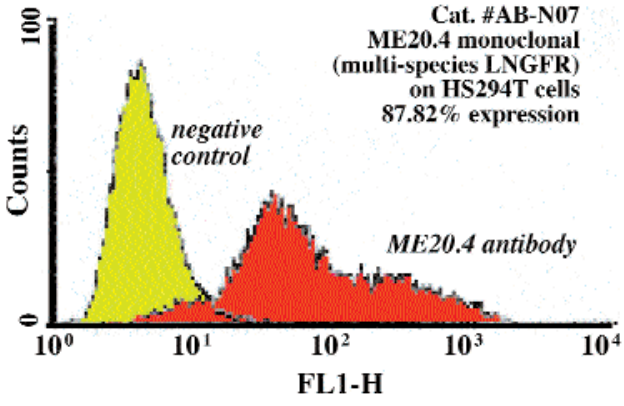
NEW!

Product Pricing
50 µl $150

ME20.4 Monoclonal and Immunotoxin

The ME20.4 monoclonal antibody was derived from immunization of mice with WM245 melanoma cells. The antibody can be used in Western blot, immunoprecipitation, immunohistochemistry, FACS analysis, and electron microscopy. The known species reactivity includes human, primate, rabbit, raccoon, dog, cat, and sheep.

The ME20.4-SAP immunotoxin is a chemical conjugation between the p75NTR monoclonal antibody and saporin, a ribosome-inactivating protein. This immunotoxin specifically eliminates p75NTR neurons in multiple species (see cover article by Dr. Beach).



NEW!

Product Pricing

Monoclonal Antibody, Cat #AB-N07
100 µg $250

Immunotoxin, Cat # IT-15
Available Individually and as a Kit *(with unconjugated antibody and saporin)*
25 µg $200, ($300)
100 µg $600, ($800)
250 µg $1200, ($1500)

Targeting Technology

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

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

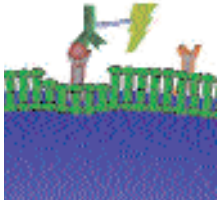
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

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

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

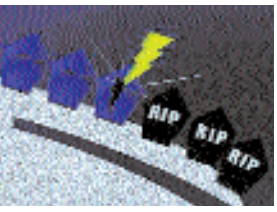
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

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



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

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



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

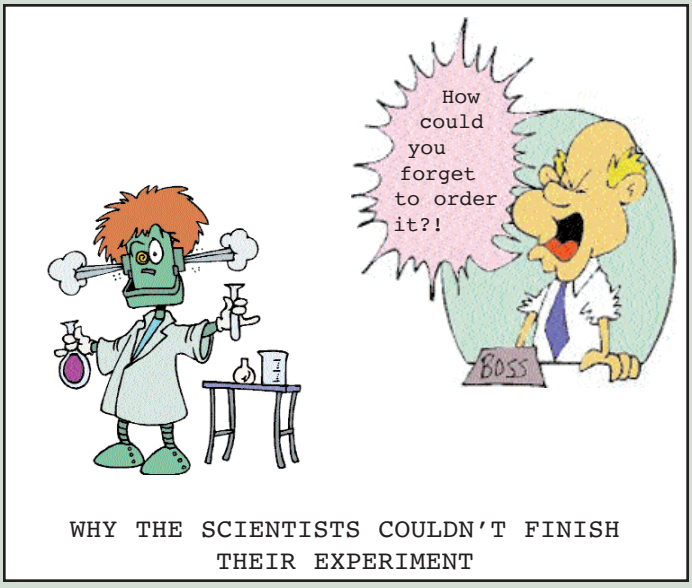
Unscramble these four Jumbles, one letter to each block, to form four words used in science.

PORSINA

RHACLEO

TLORANF

CTOKKUNO



WHY THE SCIENTISTS COULDN'T FINISH THEIR EXPERIMENT

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

THEY WERE...

Answer:

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by December 31, 2000.
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