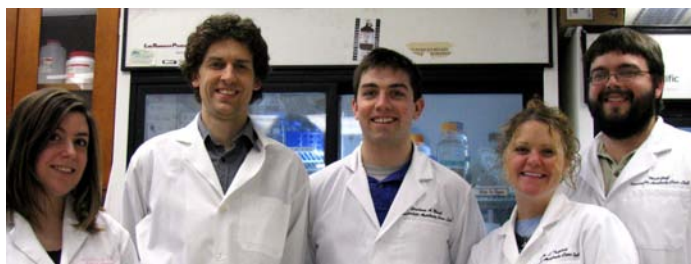


CORE NEWS: VMAC - Aggressive Pursuit of Monoclonal Antibodies

Rob Carnahan admits that when he volunteered to help Al Reynolds with the administration of the new Vanderbilt Monoclonal Antibody Core (VMAC), he knew very little about running a monoclonal antibody facility. Rob also admits that the Core had a somewhat rocky start early in 2006, leading him to spend some time in Bill Sutherland's lab (University of Virginia) to learn about the operation of a dedicated MAb production facility. With that training, and some ongoing advice from Sutherland's group, Rob was ready to turn the VMAC into a highly efficient full service operation, a goal he has achieved over the past two years.



Kimberly Cook, Rob Carnahan, Graham Black, Tracy Triplett, Matt Goff

an important role in the process. Core staff will carry out the immunizations, fusions, cloning, and initial ELISA screening, but it will be up to you to perform the secondary tests required to identify those antibodies that best suit your needs. Rob's policy is to aggressively test every promising clone, providing a level of interactive collaboration that cannot be obtained from a commercial MAb laboratory. The consequence is a few weeks of very intense screening effort, but Rob points out that this is a small price to pay for a valuable reagent that you will use for years to come.

In addition to MAb development, the VMAC performs all support services for MAb production and purification, PAb affinity purification, and hybridoma storage at highly competitive prices. The Core also has a stock of some commonly used antibodies for sale to Vanderbilt investigators. For more information, go to www.vanderbilt.edu/vmac or contact Rob at vmac@vanderbilt.edu.

Despite his administrative duties, Rob remains a scientist at heart, and he brings his enthusiasm for experimentation into VMAC operations. As a result, he is always challenging the staff to try new things, which sometimes drives them crazy. This approach has led to real benefits, however, leading to numerous improvements to antibody production. These include adoption of a new, more highly productive myeloma cell line for fusions, improvements in the growth medium, and a switch to culture in methylcellulose to improve identification and selection of productive clones. Most recently, he is experimenting with new methods of cell fusion with Dr. Jim Crowe.

If you want to engage the services of the VMAC, you can expect to play

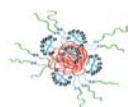
RESEARCH HIGHLIGHTS: Harth Lab Explores the Latest in Drug Delivery

One of the great challenges for 21st century drug discovery will be developing new methods for high efficiency drug delivery. This goal is imperative if the promise of newer, highly potent small molecule and more complex biological therapeutics is to be fully realized. Meeting this challenge head-on are Eva Harth and her laboratory, who are applying all of the tools of elegant polymer chemistry to the creation of versatile targeted drug delivery systems.

The first problem to tackle was how to get complex molecules into cells. Based on the observation that certain peptides containing a high arginine content easily cross cellular membranes, the Harth lab designed and synthesized a dendritic molecular transporter. This molecule, bearing nine guanidinium groups attached to a central core via hydrocarbon linkers of varying length, facilitated the rapid uptake of a covalently attached fluorescent dye into cells. The linker length dictated the subcellular localization of the transporter following uptake. More exciting, however, was the demonstration that a monoclonal antibody directed against the fusion protein of respiratory syncytial virus (RSV) could be linked to the transporter by a disulfide bond, and that this conjugate would be rapidly taken up by RSV infected cells. Intracellular disulfide bond reduction released the antibody, which effectively suppressed RSV replication.

These initial studies (conducted with Dr. Peter Wright, now at Dartmouth Medical School) suggested that the dendritic transporter may be used to develop antibody-based therapies for intracellular pathogens, which often evade immune system control.

Although the dendritic transporter entered cells very efficiently, it could only carry a single effector molecule with it at a time. So the next challenge was to develop a particle by which the transporter could carry multiple lower molecular weight species into cells at once. The first answer to this problem was a 5 - 10 nm nondegradable nanoparticle constructed from a 3-D cross-linked linear hydrophilic polymer. The multifunctionality of the polymer scaffold allowed for the attachment of a fluorescent dye (to monitor uptake), the dendritic molecular transporter (to facilitate uptake), and up to 25 model peptides (as prototypical drug molecules). Once again, the peptides were linked by disulfide bonds to allow intracellular release. These particles were rapidly taken up by cells, a process that clearly depended on the presence of the dendritic transporter. The Harth lab is presently exploring the use of these particles to deliver siRNA into cells.



Perhaps the most exciting development in the Harth lab is the construction of nanopar-

ticles formed by controlled cross-linking of polyester chains. These particles can be made in different predetermined sizes, and designed to carry varying levels of functionalities. Their amorphous state and organic solubility make them ideal for small molecule drug loading and linear, slow release. They can be modified to carry the dendritic molecular transporter as well as targeting peptides. A prototype particle loaded with Paclitaxel and bearing a peptide to target it to tumor vasculature suppressed the growth of breast cancer for up to 70 days in an animal model, as shown in preliminary experiments performed with Drs. Hallahan and Diaz (VICC). These extremely versatile particles are superior in many ways to the semi-crystalline polyester particles that have been used for drug delivery applications in the past. They offer great promise for the targeted delivery of slow release therapeutics for a wide range of applications. Check out Eva's website for more about her work and links to her recent publications at www.vanderbilt.edu/AnS/Chemistry/groups/harth.



Eva Harth

RESEARCH HIGHLIGHTS: New Approaches to Neuropsychiatric Diseases

During the 1950s doctors treating patients suffering from an overdose of the street drug phencyclidine (PCP or “angel dust”) noted that it could induce schizophrenia-like symptoms in their patients. The later discovery that PCP is a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) class of glutamate receptors led to the hypothesis that schizophrenia results from a deficit in glutamate signaling. The obvious solution to this problem - the administration of NMDA agonists - was fraught with potential pitfalls, since NMDA receptor overactivity is strongly associated with neurotoxicity. An alternative approach, which is being aggressively pursued by Jeff Conn, Craig Lindsley, and their colleagues in the Vanderbilt Program of Drug Discovery (VPDD), is to develop drugs that enhance NMDA receptor activity through modulating receptors. Possible targets include muscarinic acetylcholine receptors (mAChR) and metabotropic glutamate receptors (mGluR), both of which are known to upregulate NMDA receptor activity.



Jeff Conn

The mAChRs and mGluRs are G protein-coupled receptors (GPCRs). Like most receptors in this class, they exist as multiple subtypes of which mAChR M1 and mGluR5 have been strongly implicated as NMDA modulators. Since the various subtypes carry out distinct functions in the CNS and periphery, nonspecific activation of either mAChRs or mGluRs invariably leads to unacceptable side effects. Thus, successful mAChR M1- or mGluR5-targeted therapy requires a very high degree of subtype specificity, an elusive goal due to the conservation of orthosteric binding sites among GPCR subtypes. The VPDD group is overcoming this problem by exploiting the presence of subtype-specific allosteric binding sites, through which they can modulate receptor function.

Both mAChR M1 and mGluR5 activate calcium mobilization through Gq, providing the basis for a fluorescence-based high-throughput screen developed by the Conn lab, together with Dave Weaver in the HTS Facility. Key to the assay’s success is its ability to simultaneously identify agonists, antagonists, and allosteric potentiators. The identification of agonists or antagonists as orthosteric or allosteric requires secondary

screens employing competitive binding, and/or activation of receptors bearing mutations at the orthosteric site. Screening of both the Vanderbilt chemical library and the MLSCN library produced numerous potential hits, which have been optimized by the Lindsley group. The hit optimization process has been challenging, since small structural changes have often produced widely ranging biological effects, making rational compound design difficult. A number of compound series presented flat structure-activity relationships, with few active compounds even within a large library. Despite these difficulties, both the mAChR M1 and mGluR5 programs have produced promising compounds that exhibit antipsychotic activity in animal models.



Dave Weaver

The promise of drugs targeted to mAChR signaling has been validated by the discovery that xanomeline, an M1/M4 agonist originally developed for Alzheimer’s disease, has substantial anti-psychotic activity. Xanomeline ameliorates the “negative” symptoms of schizophrenia, such as social withdrawal, and anhedonia, that do not respond well to classic anti-psychotics. Unfortunately, the clinical use of xanomeline is limited by peripheral muscarinic side effects, highlighting the critical need for highly subtype specific drugs.

VPDD’s approach to develop mAChR M1 and mGluR5 allosteric modulators has been fruitful for the discovery of modulators of other GPCR subtypes of interest for the treatment of a range of neuropsychiatric diseases, including Parkinson’s disease, Alzheimer’s disease, dystonia, and anxiety disorders. The recent multimillion dollar commitment by Johnson & Johnson to carry one of their drug classes into clinical trials indicates the VPDD may prove to be a key player in the future of therapy for neuropsychiatric disorders. Check out the Conn lab website for more on drug discovery efforts at www.connlab.com.



Craig Lindsley

SEMINARS

The VICB Seminar Series continues into spring.
 All of the following seminars are at 12:15 PM in 1220 MRB III.

- April 01 Reinhard Sterner, University of Regensburg
- April 08 Frank Raushel, Texas A&M
- April 15 Deborah Hung, Harvard Medical School
- April 22 David Cane, Brown University
- April 29 Wolfgang Peti, Brown University

For more information, please visit: www.vanderbilt.edu/vicb/seminars2008-09.htm