

11 June 2009

This supplement has been prepared to present scientific and technical news items that may be of more interest to technical personnel at RDT&E activities and the labs, or the medics rather than the broader readership of the basic CB Daily. Due to the nature of the material, the articles, if available online, are usually only available through subscription services thus making specific links generally unavailable. Thus, usually only the bibliographic citation is available for use by an activity's technical library.

Should you wish to be removed from this S&T Supplement address group, just send an email to one of the people listed at the bottom of this message. This will not affect your continued receipt of the CB Daily

Chem-Bio News – S&T Edition

1. SELECTIVE TOXIN SEQUESTRANTS FOR THE TREATMENT OF BACTERIAL INFECTIONS:

"From 100000 compounds, we discovered a single sequence of residues that can bind and retain cholera toxin at high affinity when immobilized on a solid-phase particle."

2. CRYSTAL STRUCTURE OF THE ENGINEERED NEUTRALIZING ANTIBODY M18 COMPLEXED TO DOMAIN 4 OF THE ANTHRAX PROTECTIVE ANTIGEN:

"Here, we report the high-resolution X-ray structures of three high-affinity, single-chain antibodies in the 14B7 family; 14B7 and two high-affinity variants 1H and M18. In addition, we present the first neutralizing antibody-PA structure, M18 in complex with PAD4 at 3.8 angstrom resolution."

3. COMPARISON OF THE OXIME-INDUCED REACTIVATION OF RHESUS MONKEY, SWINE AND GUINEA PIG ERYTHROCYTE ACETYLCHOLINESTERASE FOLLOWING INHIBITION BY SARIN OR PARAOXON, USING A PERFUSION MODEL FOR THE REAL-TIME DETERMINATION OF MEMBRANE-BOUND ACETYLCHOLINESTE:

"Hence, this dynamic model offers the possibility to investigate highly reproducible interactions between AChE, OP and oximes with human and animal AChE."

4. BACILLUS ANTHRACIS EDEMA TOXIN SUPPRESSES HUMAN MACROPHAGE PHAGOCYTOSIS AND CYTOSKELETAL REMODELING VIA THE PROTEIN KINASE A AND EXCHANGE PROTEIN ACTIVATED BY CYCLIC AMP PATHWAYS:

"These results suggested that EdTx weakened the host immune response by increasing cAMP levels, which then signaled via PKA and Epac to cripple MPhi phagocytosis and interfered with cytoskeletal remodeling."

5. CYCLOPHILIN A FACILITATES TRANSLOCATION OF THE CLOSTRIDIUM BOTULINUM C2 TOXIN ACROSS MEMBRANES OF ACIDIFIED ENDOSOMES INTO THE CYTOSOL OF MAMMALIAN CELLS:

"Our results suggest an essential role of cyclophilin A for translocation of C2I across endosomal membranes during the uptake of C2 toxin into mammalian cells."

6. BACILLUS ANTHRACIS EDEMA TOXIN IMPAIRS NEUTROPHIL ACTIN-BASED MOTILITY:

"We conclude that ET alone or combined with LT impairs PMN actin assembly, resulting in paralysis of PMN chemotaxis."

7. DEVELOPMENT AND USE OF A CHROMOGENIC MACROARRAY SYSTEM FOR THE DETECTION OF STAPHYLOCOCCUS AUREUS WITH ENTEROTOXIN A, B, C, D, E, AND G GENES IN FOOD AND MILK SAMPLES:

"This macroarray offers clinical and food inspection laboratories a rapid and economical visual method to detect common enterotoxigenic S. aureus strains."

8. CULTURING RARE MICROBES:

"Getting microbes such as Escherichia coli to grow may be easy enough, but what if you want to amplify the rarer, slower-growing species within a microbe mixture? US biochemists are using microfluidics to do just this."

9. SCIENTISTS USE CLIMATE VARIABLES AND VEGETATION INDICES TO PREDICT AND MITIGATE DENGUE EPIDEMICS:

"The new model can predict Dengue Fever epidemics with 83% accuracy, up to 40 weeks in advance of an outbreak and provide information on the magnitude of future epidemics."

10. ANTIBACTERIAL NANOTECHNOLOGY MULTI-ACTION MATERIALS THAT WORK DAY AND NIGHT:

"Researchers in China have now further advanced the nanotechnology application of silver by developing a novel multi-action nanofiber membrane containing four active components, each playing a different role in the membrane's excellent antibacterial function."

CB Daily Report

Chem-Bio News

SELECTIVE TOXIN SEQUESTRANTS FOR THE TREATMENT OF BACTERIAL INFECTIONS

Gastroenterology Week

June 1, 2009

"In this work we designed an on-bead library of protease-resistant, acid-stable peptoid molecules and screened for high affinity binding of cholera toxin."

"From 100000 compounds, we discovered a single sequence of residues that can bind and retain cholera toxin at high affinity when immobilized on a solid-phase particle. Furthermore, we demonstrate that these peptoid-displaying particles can sequester active cholera toxin from cell culture media sufficient to protect intestinal cells."

The full article can be found at: (L.S. Simpson, et. al., "Selective Toxin Sequestrants for the Treatment of Bacterial Infections". Journal of the American Chemical Society, 2009;131(16):5760+). Link not available.

[Return to Top](#)

CRYSTAL STRUCTURE OF THE ENGINEERED NEUTRALIZING ANTIBODY M18 COMPLEXED TO DOMAIN 4 OF THE ANTHRAX PROTECTIVE ANTIGEN

Medical Letter on the CDC & FDA

June 14, 2009

"The virulence of Bacillus anthracis is critically dependent on the cytotoxic components of the anthrax toxin, lethal factor (LF) and edema factor (EF). LF and EF gain entry into host cells through interactions with the protective antigen (PA), which binds to host cellular receptors such as CMG2."

"Antibodies that neutralize PA have been shown to confer protection in animal models and are undergoing intense clinical development. A murine monoclonal antibody, 14B7, had been reported to interact with domain 4 of PA (PAD4) and block its binding of CMG2. More recently, the 14B7 antibody was used as the platform for the selection of very high affinity, single-chain antibodies that have tremendous potential as a combination anthrax prophylactic and treatment. Here, we report the high-resolution X-ray structures of three high-affinity, single-chain antibodies in the 14B7 family; 14B7 and two high-affinity variants 1H and M18. In addition, we present the first neutralizing antibody-PA structure, M18 in complex with PAD4 at 3.8 angstrom resolution."

"These structures provide insights into the mechanism of neutralization, and the effect of various mutations on antibody affinity, and enable a comparison between the binding of the M18 antibody and CMG2 with PAD4."

The full article can be found at: (C.E. Leysath, et. al., "Crystal Structure of the Engineered Neutralizing Antibody M18 Complexed to Domain 4 of the Anthrax Protective Antigen". Journal of Molecular Biology, 2009;387(3):680-693). Link not available.

[Return to Top](#)

COMPARISON OF THE OXIME-INDUCED REACTIVATION OF RHESUS MONKEY, SWINE AND GUINEA PIG ERYTHROCYTE ACETYLCHOLINESTERASE FOLLOWING INHIBITION BY SARIN OR

PARAOXON, USING A PERFUSION MODEL FOR THE REAL-TIME DETERMINATION OF MEMBRANE-BOUND ACETYLCHOLINESTERASE

Life Science Weekly
June 2, 2009

"Recently, a dynamically working in vitro model with real-time determination of membrane-bound human acetylcholinesterase (AChE) activity was shown to be a versatile model to investigate oxime-induced reactivation kinetics of organophosphate-(OP) inhibited enzyme. In this assay, AChE was immobilized on particle filters which were perfused with acetylthiocholine, Ellman's reagent and phosphate buffer."

Subsequently, AChE activity was continuously analyzed in a flow-through detector. Now, it was an intriguing question whether this model could be used with erythrocyte AChE from other species in order to investigate kinetic interactions in the absence of annoying side reactions. Rhesus monkey, swine and guinea pig erythrocytes were a stable and highly reproducible enzyme source. Then, the model was applied to the reactivation of sarin- and paraoxon-inhibited AChE by obidoxime or HI 6 and it could be shown that the derived reactivation rate constants were in good agreement to previous results obtained from experiments with a static model."

"Hence, this dynamic model offers the possibility to investigate highly reproducible interactions between AChE, OP and oximes with human and animal AChE."

The full article can be found at: (N.M. Herkert, et. al., "Comparison of the oxime-induced reactivation of rhesus monkey, swine and guinea pig erythrocyte acetylcholinesterase following inhibition by sarin or paraoxon, using a perfusion model for the real-time determination of membrane-bound acetylcholinesterase". Toxicology, 2009;258(2-3):79-83). Link not available.

[Return to Top](#)

BACILLUS ANTHRACIS EDEMA TOXIN SUPPRESSES HUMAN MACROPHAGE PHAGOCYTOSIS AND CYTOSKELETAL REMODELING VIA THE PROTEIN KINASE A AND EXCHANGE PROTEIN ACTIVATED BY CYCLIC AMP PATHWAYS

Health & Medicine Week
June 1, 2009

"Bacillus anthracis edema toxin suppresses human macrophage phagocytosis and cytoskeletal remodeling via the protein kinase A and exchange protein activated by cyclic AMP pathways,' are detailed in a study published in Infection and Immunity. According to recent research from the United States, "Bacillus anthracis, the etiological agent of anthrax, is a gram-positive spore-forming bacterium. It produces edema toxin (EdTx), a powerful adenylate cyclase that increases cyclic AMP (cAMP) levels in host cells."

"Because other cAMP-increasing agents inhibit key macrophage (MPhi) functions, such as phagocytosis, it was hypothesized that EdTx would exhibit similar suppressive activities. Our previous GeneChip data showed that EdTx downregulated MPhi genes involved in actin cytoskeleton remodeling, including protein kinase A (PKA). To further examine the role of EdTx during anthrax pathogenesis, we explored the hypothesis that EdTx treatment leads to deregulation of the cAMP-dependent PKA system, resulting in impaired cytoskeletal functions essential for MPhi activity. Our data revealed that EdTx significantly suppressed human MPhi phagocytosis of Ames spores. Cytoskeletal changes, such as decreased cell spreading and lowered F-actin content, were also observed for toxin-treated MPhis. Further, EdTx altered the protein levels and activity of PKA and exchange protein activated by cAMP (Epac), a recently identified cAMP-binding molecule. By using PKA-and Epac-selective cAMP analogs, we confirmed the involvement of both pathways in the inhibition of MPhi functions elicited by EdTx-generated cAMP."

"These results suggested that EdTx weakened the host immune response by increasing cAMP levels, which then signaled via PKA and Epac to cripple MPhi phagocytosis and interfered with cytoskeletal remodeling."

The full article can be found at: (L.A. Yeager, et. al., "Bacillus anthracis edema toxin suppresses human macrophage phagocytosis and cytoskeletal remodeling via the protein kinase A and exchange protein activated by cyclic AMP pathways. *Infection and Immunity*, 2009;77(6):2530-43). Link not available.

[Return to Top](#)

CYCLOPHILIN A FACILITATES TRANSLOCATION OF THE CLOSTRIDIUM BOTULINUM C2 TOXIN ACROSS MEMBRANES OF ACIDIFIED ENDOSOMES INTO THE CYTOSOL OF MAMMALIAN CELLS

Biotech Week

June 10, 2009

"The binary *Clostridium botulinum* C2 toxin consists of the binding/translocation component C2IIa and the separate enzyme component C2I, which mono-ADP-ribosylates actin in eukaryotic cells. Pore formation of C2IIa in early endosomal membranes facilitates translocation of unfolded C2I into the cytosol."

"We discovered earlier that translocation of C2I depends on the activity of the host cell chaperone heat shock protein Hsp90. Here, we demonstrate that cyclosporin A, which inhibits the peptidyl-prolyl cis/trans isomerase activity of cyclophilins, inhibited intoxication of cells with C2 toxin and prevented uptake of C2I into the cytosol. Cyclosporin A blocked the pH-dependent translocation of C2I activity across membranes of intact cells and of partially purified early endosomes. In vitro, the addition of cytosol to C2 toxin-loaded endosomes induced translocation of C2I activity into the cytosol, which was prevented by pretreatment of the cytosol with an antibody against cyclophilin A. Pull-down experiments with lysates from C2 toxin-treated cells revealed specific binding of cyclophilin A to the N-terminal domain of C2I."

"Our results suggest an essential role of cyclophilin A for translocation of C2I across endosomal membranes during the uptake of C2 toxin into mammalian cells."

The full article can be found at: (E. Kaiser, et. al., "Cyclophilin A facilitates translocation of the *Clostridium botulinum* C2 toxin across membranes of acidified endosomes into the cytosol of mammalian cells". *Cellular Microbiology*, 2009;11(5):780-795). Link not available.

[Return to Top](#)

BACILLUS ANTHRACIS EDEMA TOXIN IMPAIRS NEUTROPHIL ACTIN-BASED MOTILITY

Health & Medicine Week

June 1, 2009

"Relatively low concentrations of ET (100 to 500 ng/ml of PA and EF) significantly impair human PMN chemokinesis, chemotaxis, and ability to polarize. These changes are accompanied by a reduction in chemoattractant-stimulated PMN actin assembly. ET also causes a significant decrease in *Listeria monocytogenes* intracellular actin-based motility within HeLa cells. These defects in actin assembly are accompanied by a 50-fold increase in intracellular cyclic AMP and a 4-fold increase in the phosphorylation of protein kinase A. We have previously shown that anthrax lethal toxin (LT) also impairs neutrophil actin-based motility (R. L. During, W. Li, B. Hao, J. M. Koenig, D. S. Stephens, C. P. Quinn, and F. S. Southwick, *J. Infect. Dis.* 192:837-845, 2005), and we now find that LT combined with ET causes an additive inhibition of PMN chemokinesis, polarization, chemotaxis, and FMLP (N-formyl-met-leu-phe)-induced actin assembly."

"We conclude that ET alone or combined with LT impairs PMN actin assembly, resulting in paralysis of PMN chemotaxis."

The full article can be found at: (S.E. Szarowicz, et. al., "Bacillus anthracis edema toxin impairs neutrophil actin-based motility". *Infection and Immunity*, 2009;77(6):2455-64). Link not available.

[Return to Top](#)

DEVELOPMENT AND USE OF A CHROMOGENIC MACROARRAY SYSTEM FOR THE DETECTION OF STAPHYLOCOCCUS AUREUS WITH ENTEROTOXIN A, B, C, D, E, AND G GENES IN FOOD AND MILK SAMPLES

Food Weekly News

June 11, 2009

"Two sets of degenerated primers labeled with biotin were used to co-amplify all SE genes in *S. aureus* strains through the polymerase chain reaction (PCR). Afterwards, these biotin-labeled PCR products were hybridized with SE gene-specific probes spotted on the nitrocellulose membrane. When this macroarray was used to detect enterotoxigenic *S. aureus* in milk or beef homogenate containing 10(0)-10(4) target cells per milliliter or gram of the sample, all six enterotoxin genes could be identified after a 12-hour enrichment step."

"This macroarray offers clinical and food inspection laboratories a rapid and economical visual method to detect common enterotoxigenic *S. aureus* strains."

The full article can be found at: (Development and Use of a Chromogenic Macroarray System for the Detection of *Staphylococcus aureus* with Enterotoxin A, B, C, D, E, and G Genes in Food and Milk Samples. *Foodborne Pathogens and Disease*, 2009;6(4):445-452). Link not available.

[Return to Top](#)

CULTURING RARE MICROBES

By David Barden

Chemical Biology

June 01, 2009

"Getting microbes such as *Escherichia coli* to grow may be easy enough, but what if you want to amplify the rarer, slower-growing species within a microbe mixture? US biochemists are using microfluidics to do just this.

Microbe mixtures occur widely in nature, from soils and oceans to animals, making studying them of interest in several scientific fields. Led by Rustem Ismagilov, a team at the University of Chicago has developed an approach to analyse such mixtures. The method involves mixing an aqueous cell suspension with an organic solvent. This mixture is passed through a series of tubes that splits the aqueous suspension into successively smaller droplets within the solvent, ultimately producing droplets containing just one cell. It is then possible, says Ismagilov, to isolate just those droplets containing the rarer cells. These can then be grown in cultures."

The full article can be found at:

http://www.rsc.org/Publishing/Journals/cb/Volume/2009/7/Culturing_microbes.asp

[Return to Top](#)

SCIENTISTS USE CLIMATE VARIABLES AND VEGETATION INDICES TO PREDICT AND MITIGATE DENGUE EPIDEMICS

The Medical News

June 05, 2009

"Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) are the most important vector-borne viral diseases in the World. Around 50-100 million cases appear each year putting 2.5 billion people at risk of suffering this debilitating and sometimes fatal disease.

Dengue Fever is prevalent in the Tropics. For that reason, an interdisciplinary team of researchers from the University of Miami (UM) and the University of Costa Rica have used global climatological data and

vegetation indices from Costa Rica, to predict Dengue outbreaks in the region.

The new model can predict Dengue Fever epidemics with 83% accuracy, up to 40 weeks in advance of an outbreak and provide information on the magnitude of future epidemics. The model can be expanded to include the broader region of Latin America and the Caribbean, where incidence and spread of the disease has increased dramatically over the past 25 years."

The full article can be found at: <http://www.news-medical.net/news/20090605/Scientists-use-climate-variables-and-vegetation-indices-to-predict-and-mitigate-Dengue-epidemics.aspx>

[Return to Top](#)

ANTIBACTERIAL NANOTECHNOLOGY MULTI-ACTION MATERIALS THAT WORK DAY AND NIGHT

By Michael Berger

Nanowerk.com

June 02, 2009

"Researchers in China have now further advanced the nanotechnology application of silver by developing a novel multi-action nanofiber membrane containing four active components, each playing a different role in the membrane's excellent antibacterial function.

While the preparation of multicomponent materials is not new, their fabrication usually is based on the co-precipitation method where the resulting materials are generally obtained in powder form – which makes their practical application difficult because it requires further steps to prepare coatings or thin films. The novelty of the Chinese team's approach is the development of a facile and effective approach to produce membrane or film materials with comparable or even higher antibacterial activity.

"Using an electrospinning technique, we have prepared a new kind of free-standing antibacterial membranes, which contain silver, silver bromide, titanium dioxide, and hydroxyapatite as four active components," Gunagtao Li tells Nanowerk. "In this antibacterial membrane, each component serves a different function: apatite as the adsorption material for capturing bacteria, silver nanoparticles as the release-active antibacterial agent, silver bromide nanoparticles as the visible sensitive and release-active antibacterial agent, and titanium dioxide as the UV sensitive antibacterial material and substrate for other functional components."

The full article can be found at: <http://www.nanowerk.com/spotlight/spotid=10951.php>

[Return to Top](#)

END of CB Daily Report.

Send subscription requests, unsubscribing requests, questions and comments to:

Steve Tesko: Steve.Tesko@anser.org

Copyright 2008. *Analytic Services Inc.*

[Analytic Services Inc. DMCA Copyright Notice: http://www.homelandsecurity.org/bulletin/Draft_ANSER_DCMA_Copyright_Notice.htm](http://www.homelandsecurity.org/bulletin/Draft_ANSER_DCMA_Copyright_Notice.htm)

Use of these news articles does not reflect official endorsement.
In accordance with Title 17 (USC), Section 107, this material is distributed without profit or payment and is intended for nonprofit research and educational purposes only.

Reproduction for private use or gain is subject to original copyright restrictions.

PRIVACY POLICY

Content provided in the *CB Daily Report* does not reflect the viewpoint(s) of Analytic Services Inc. Analytic Services Inc. does not share, publish, or in any way redistribute subscriber email addresses or any other personal information.