

7 August 2008

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 Supplement

1. KILLING OF MACROPHAGES BY ANTHRAX LETHAL TOXIN [LT]: INVOLVEMENT OF

THE N-END RULE PATHWAY: *"We also show that LT-induced c-IAP1 degradation is independent of the IAP-antagonizing proteins Smac/DIABLO and Omi/HtrA2, but dependent on caspases."*

2. TRANSASIA ANNOUNCES ALL INDIA LAUNCH OF ERBA DEN-GO TO DETECT

DENGUE: *"This rapid test is designed to simultaneously detect and differentiate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood and comes in packing of 15 tests per box."*

3. SITE-DIRECTED MUTAGENESIS OF THE HINGE PEPTIDE FROM THE HEMAGGLUTININ PROTEIN: ENHANCEMENT OF THE PH-RESPONSIVE

CONFORMATIONAL CHANGE: *"Therefore this mutant stimulus-responsive peptide may be more valuable for future protein engineering and bionanotechnology efforts."*

4. NEXT GENERATION TOOL FOR VISUALIZING GENOMIC DATA INTRODUCED:

"Researchers are collecting vast amounts of diverse genomic data with ever-increasing speed, but effective ways to visualize these data in an integrated manner have lagged behind the ability to generate them."

5. SCIENTISTS IDENTIFY SEVERAL HUNDRED GENES THAT IMPACT WEST NILE

VIRUS INFECTION: *"West Nile virus consists of only 10 proteins so it must hijack dozens of cellular processes of the host in order to infect individuals and replicate."*

6. IMMUNOVACCINE WINS JAPANESE PATENT FOR VACCINE TECHNOLOGY:

"The specific patent, vaccines with enhanced immune response and methods for their preparation, claims broad exclusivity that an effective, long-term immune response to treat a disease can be produced using a vaccine comprising of an antigen, an adjuvant, vesicles known as liposomes, and a hydrophobic carrier."

7. STRUCTURE OF THE EBOLA VIRUS GLYCOPROTEIN BOUND TO AN ANTIBODY

FROM A HUMAN SURVIVOR: *"This structure provides a template for unravelling the mechanism of EBOV GP- mediated fusion and for future immunotherapeutic development."*

8. DETECTION OF STAPHYLOCOCCUS ENTEROTOXIN B [SEB] USING FLUORESCENT IMMUNOLIPOSOMES AS LABEL FOR IMMUNOCHROMATOGRAPHIC TESTING:

"The ICT [immunochromatographic test] was completed within 30 min, providing a limit of detection close to 20 pg/ml in buffer and showing no cross-reactivity with the other major toxin of the bacterium, Staphylococcus enterotoxin A."

9. IDENTIFICATION OF RESIDUES SURROUNDING THE ACTIVE SITE OF TYPE A BOTULINUM NEUROTOXIN IMPORTANT FOR SUBSTRATE RECOGNITION AND CATALYTIC ACTIVITY:

"We suggest that, despite the large minimal substrate size for catalysis, only a few non-conserved residues surrounding the active site are important to render the LC competent for catalysis or provide conformational selection of the substrate."

10. THE USE OF DOXYCYCLINE AS A PROTECTANT AGAINST SULPHUR MUSTARD IN HACAT CELLS:

"The results suggested that doxycycline and other MMP[matrix metalloproteinase] inhibitors may have a role to play in therapeutic intervention against HD exposure, but only as part of a combination therapy."

11. DETERMINATION OF BASIC DEGRADATION PRODUCTS OF CHEMICAL WARFARE AGENTS IN WATER USING HOLLOW FIBRE-PROTECTED LIQUID-PHASE MICROEXTRACTION WITH IN-SITU DERIVATISATION FOLLOWED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY:

"The limits of detection of HF-LPME (0.04-0.36 microg l(-1)) showed improvement over those of SPME (0.06-0.77 microg l(-1))."

CB Daily Report

Chem-Bio News

KILLING OF MACROPHAGES BY ANTHRAX LETHAL TOXIN [LT]: INVOLVEMENT OF THE N-END RULE PATHWAY

Pharma Investments, Ventures & Law Weekly
August 10, 2008

"Proteasome inhibitors block LT-mediated caspase-1 activation and can protect against cell death, indicating that the degradation of at least one cellular protein is required for LT-mediated cell death. Proteins can be degraded by the proteasome via the N-end rule, in which a protein's stability is determined by its N-terminal residue. Using amino acid derivatives that act as inhibitors of this pathway, we show that the N-end rule is required for LT-mediated caspase-1 activation and cell death. We also found that bestatin methyl ester, an aminopeptidase inhibitor protects against LT in vitro and in vivo and that the different inhibitors of the protein degradation pathway act synergistically in protecting against LT. We identify c-IAP1, a mammalian member of the inhibitor of apoptosis protein (IAP) family, as a novel N-end rule substrate degraded in macrophages treated with LT."

"We also show that LT-induced c-IAP1 degradation is independent of the IAP-antagonizing proteins Smac/DIABLO and Omi/HtrA2, but dependent on caspases."

The full article can be found at: Cellular Microbiology (K.E. Wickliffe, et. al., "Killing of macrophages by anthrax lethal toxin: involvement of the N-end rule pathway". Cellular Microbiology, 2008; 10(6):1352-1362). Link not available.

[Return to Top](#)

TRANSASIA ANNOUNCES ALL INDIA LAUNCH OF ERBA DEN-GO TO DETECT DENGUE

The Financial Express [India]

August 1, 2008

"To provide a user-friendly and rapid diagnostic method for detection of dengue infection, Transasia Bio-Medicals Ltd has launched its brand new product, Erba Den-GO (CE marked) this shower season!

The key advantages of this new test are that it is a 15 minute procedure. The test allows presumptive differentiation between primary and secondary dengue with an overall accuracy of 99.3%."

"This rapid test is designed to simultaneously detect and differentiate IgG and IgM antibodies to dengue virus in human serum, plasma or whole blood and comes in packing of 15 tests per box."

The full article can be found at: <http://www.financialexpress.com/news/Transasia-announces-all-India-launch-of-Erba-DenGo-to-detect-dengue/343427/>

[Return to Top](#)

SITE-DIRECTED MUTAGENESIS OF THE HINGE PEPTIDE FROM THE HEMAGGLUTININ PROTEIN: ENHANCEMENT OF THE PH-RESPONSIVE CONFORMATIONAL CHANGE

Biotech Week

August 6, 2008

"Environmentally responsive proteins and peptides are increasingly finding utility in various engineered systems due to their ability to respond to the presentation of external stimuli. A classic example of this behavior is the influenza hemagglutinin (HA) fusion protein."

"At neutral pH, HA exists in a non-fusogenic state, but upon exposure to low pH, the conformation of the structure changes to expose a fusogenic peptide. During this structural change, massive rearrangements occur in a subunit of HA (HA2). Crystallography data has shown that a loop of 28 amino acids (residues 54-81) undergoes a dramatic transition from a random coil to an alpha-helix. This segment connects to two flanking helical regions (short

and long) to form a long, continuous helix. Here, we report the results of site-directed mutagenesis study on LOOP-36 to further understand the mechanism of this important stimulus-responsive peptide. The conformational transition of a bacterially expressed LOOP-36 was found to be less dramatic than has been previously reported. The systematic mutation of glutamate and histidine residues in the peptide to glutamines (glutamine scanning) did not impact the conformational behavior of the peptide, but the substitution of the glycine residue at position 22 with alanine resulted in significant pH-responsive behavior."

"Therefore this mutant stimulus-responsive peptide may be more valuable for future protein engineering and bionanotechnology efforts."

The full article can be found at: Protein Engineering, Design & Selection (M. Casali, et. al., "Site-directed mutagenesis of the hinge peptide from the hemagglutinin protein: enhancement of the pH-responsive conformational change". Protein Engineering, Design & Selection, 2008; 21(6): 395-404). Link not available.

[Return to Top](#)

NEXT GENERATION TOOL FOR VISUALIZING GENOMIC DATA INTRODUCED

ScienceDaily

August 6, 2008

"Researchers are collecting vast amounts of diverse genomic data with ever-increasing speed, but effective ways to visualize these data in an integrated manner have lagged behind the ability to generate them.

To address this growing need, researchers at the Broad Institute have developed the Integrative Genomics Viewer (IGV), a novel and freely available visualization tool that helps users simultaneously integrate and analyze different types of genomic data, and gives them the flexibility to zoom in on a specific genomic region of interest or to pan out for a broad, whole genome view.

"This new tool offers a Google Maps-like view of integrative genomic data," said Jill Mesirov, Chief Informatics Officer and Director of Computational Biology and Bioinformatics at the Broad Institute. "It brings together different kinds of genomic data into a single, holistic view. I'm incredibly proud of our computational scientists for responding so rapidly and effectively to the critical needs of the growing genomics research community."

The full article can be found at: <http://www.sciencedaily.com/releases/2008/08/080804111644.htm>

The IGV website can be found at: <http://www.broad.mit.edu/igv/>

[Return to Top](#)

SCIENTISTS IDENTIFY SEVERAL HUNDRED GENES THAT IMPACT WEST NILE VIRUS INFECTION

News-Medical.net

August 6, 2008

"West Nile virus consists of only 10 proteins so it must hijack dozens of cellular processes of the host in order to infect individuals and replicate. To find out exactly which of those processes were involved in an infection, the team from Yale and three other research institutions used a technique called global RNA interference targeting strategy.

Using tiny snippets of small interfering RNA, scientists are now able to disable individual genes and thereby assess their function. Testing the entire human genome, the team was able to identify 305 individual proteins that can alter viral infection. Many of those proteins appear crucial to the ability of the virus to infect people and reproduce. About 30 percent of the genes involved in West Nile infection also appear to play a role in Dengue fever, the researchers report."

The full article can be found at: <http://www.news-medical.net/?id=40570>

[Return to Top](#)

IMMUNOVACCINE WINS JAPANESE PATENT FOR VACCINE TECHNOLOGY

Pharmaceutical Business Review

August 6, 2008

"The specific patent, vaccines with enhanced immune response and methods for their preparation, claims broad exclusivity that an effective, long-term immune response to treat a disease can be produced using a vaccine comprising of an antigen, an adjuvant, vesicles known as liposomes, and a hydrophobic carrier."

The full article can be found at: http://www.pharmaceutical-business-review.com/article_news.asp?guid=DB921AF9-5F69-4293-8B8F-A9A6F3CE9A12

[Return to Top](#)

STRUCTURE OF THE EBOLA VIRUS GLYCOPROTEIN BOUND TO AN ANTIBODY FROM A HUMAN SURVIVOR

Virus Weekly

August 12, 2008

"Ebola virus (EBOV) entry requires the surface glycoprotein (GP) to initiate attachment and fusion of viral and host membranes. Here we report the crystal structure of EBOV GP in its trimeric, pre- fusion conformation (GP1+GP2) bound to a neutralizing antibody, KZ52, derived from a human survivor of the 1995 Kikwit outbreak."

"Three GP1 viral attachment subunits assemble to form a chalice, cradled by the GP2 fusion subunits, while a novel glycan cap and projected mucin- like domain restrict access to the conserved receptor- binding site sequestered in the chalice bowl. The glycocalyx surrounding GP is likely central to immune evasion and may explain why survivors have insignificant neutralizing antibody titres. KZ52 recognizes a protein epitope at the chalice base where it clamps several regions of the pre- fusion GP2 to the amino terminus of GP1."

"This structure provides a template for unravelling the mechanism of EBOV GP- mediated fusion and for future immunotherapeutic development."

The full article can be found at: Nature (J.E. Lee, et. al. "Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor". Nature, 2008; 454(7201): 177- U27). Link not available.

[Return to Top](#)

DETECTION OF STAPHYLOCOCCUS ENTEROTOXIN B [SEB] USING FLUORESCENT IMMUNOLIPOSOMES AS LABEL FOR IMMUNOCHROMATOGRAPHIC TESTING

TB & Outbreaks Week

August 12, 2008

"Staphylococcus enterotoxin B (SEB) is one of several toxins produced by the gram positive bacterium Staphylococcus aureus. SEB is a major cause of food poisoning and represents a significant biological threat with regard to bioterrorism."

"A rapid, sensitive, and specific method is required to monitor food and water in cases of both natural and intentional contamination by this toxin. This report presents an improved immunochromatographic test (ICT) using immunoliposomes as label for the detection of SEB. For the first time in an ICT, the signal generated by the sulforhodamine B encapsulated into immunoliposomes was measured by fluorescence, allowing a 15-fold increase in sensitivity compared with that for visual detection of colored labels."

"The ICT was completed within 30 min, providing a limit of detection close to 20 pg/ml in buffer and showing no cross-reactivity with the other major toxin of the bacterium, Staphylococcus enterotoxin A. This sensitivity was retained when analyzing SEB spiked in various alimentary matrices, mimicking contaminated foods."

The full article can be found at: Analytical Biochemistry (N. Khreich, et. al., "Detection of Staphylococcus enterotoxin B using fluorescent immunoliposomes as label for immunochromatographic testing". Analytical Biochemistry, 2008; 377(2): 182-188). Link not available.

[Return to Top](#)

IDENTIFICATION OF RESIDUES SURROUNDING THE ACTIVE SITE OF TYPE A BOTULINUM NEUROTOXIN IMPORTANT FOR SUBSTRATE RECOGNITION AND CATALYTIC ACTIVITY

Drug Week

August 15, 2008

"Type A botulinum neurotoxin is one of the most lethal of the seven serotypes and is increasingly used as a therapeutic agent in neuromuscular dysfunctions. Its toxic function is related to zinc-endopeptidase activity of the N-terminal light chain (LC) on synaptosome-associated protein-25 kDa (SNAP-25) of the SNARE complex."

"To understand the determinants of substrate specificity and assist the development of strategies for effective inhibitors, we used site-directed mutagenesis to investigate the effects of 13 polar residues of the LC on substrate binding and catalysis. Selection of the residues for mutation was based on a computational analysis of the three-dimensional structure of the LC modeled with a 17-residue substrate fragment of SNAP-25. Steady-state kinetic parameters for proteolysis of the substrate fragment were determined for a set of 16 single mutants. Of the mutated residues non-conserved among the serotypes, replacement of Arg-230 and Asp-369 by polar or apolar residues resulted in drastic lowering of the catalytic rate constant ($k(\text{cat})$), but had less effect on substrate affinity (K_m). Substitution of Arg-230 with Lys decreased the catalytic efficiency ($k(\text{cat})/K_m$) by 50-fold, whereas replacement by Leu yielded an inactive protein. Removal of the electrostatic charge at Asp-369 by mutation to Asn resulted in 140-fold decrease in $k(\text{cat})/K_m$. Replacement of other variable residues surrounding the catalytic cleft (Glu-54, Glu-63, Asn-66, Asp-130, Asn-161, Glu-163, Glu-170, Glu-256), had only marginal effect on decreasing the catalytic efficiency, but unexpectedly the substitution of Lys-165 with Leu resulted in fourfold increase in $k(\text{cat})/K_m$. For comparison purposes, two conserved residues Arg-362 and Tyr-365 were investigated with substitutions of Leu and Phe, respectively, and their catalytic efficiency decreased 140- and 10-fold, respectively, whereas substitution of the tyrosine ring with Asn abolished activity. The altered catalytic efficiencies of the mutants were not due to any significant changes in secondary or tertiary structures, or in zinc content and thermal stability."

"We suggest that, despite the large minimal substrate size for catalysis, only a few non-conserved residues surrounding the active site are important to render the LC competent for catalysis or provide conformational selection of the substrate."

The full article can be found at: Protein Journal (S.A. Ahmed, et. al., "Identification of residues surrounding the active site of type a botulinum neurotoxin important for substrate recognition and catalytic activity". Protein Journal, 2008; 27(3): 151-162). Link not available.

[Return to Top](#)

THE USE OF DOXYCYCLINE AS A PROTECTANT AGAINST SULPHUR MUSTARD IN HACAT CELLS

Drug Week

August 15, 2008

"As part of an ongoing programme on medical countermeasures against the chemical warfare agent sulphur mustard (HD) and set against the background of the involvement of matrix metalloproteinases (MMPs) in the pathology of HD-induced vesication processes, the potentially beneficial effects of doxycycline on cell attachment was determined in confluent HaCaT cell cultures exposed to HD. Doxycycline was found to inhibit to a significant extent the tendency of HD-exposed cells to detach from the growth substrate, however, analysis of the metabolic activity of the adherent cells indicated that doxycycline treatment did not maintain cell viability."

"It was confirmed that apoptosis was the predominant mode of HD-induced cell death. The results suggested that doxycycline and other MMP inhibitors may have a role to play in therapeutic intervention against HD exposure, but only as part of a combination therapy."

The full article can be found at: Journal of Applied Toxicology (C.D. Lindsay, et. al., "The use of doxycycline as a protectant against sulphur mustard in HaCaT cells". Journal of Applied Toxicology, 2008;28(5):665-73). Link not available.

[Return to Top](#)

DETERMINATION OF BASIC DEGRADATION PRODUCTS OF CHEMICAL WARFARE AGENTS IN WATER USING HOLLOW FIBRE-PROTECTED LIQUID-PHASE MICROEXTRACTION WITH IN-SITU DERIVATISATION FOLLOWED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Medical Devices & Surgical Technology Week
August 17, 2008

"According to recent research from Singapore, Singapore, "Hollow fibre-protected liquid-phase microextraction (HF-LPME) together with gas chromatography-mass spectrometry was, for the first time, investigated for the in-situ derivatisation and analysis of basic degradation products of chemical warfare agents in water samples. The degradation products studied were those of nerve and blister agents, and a psychotomimetic agent."

"Extractions with in-situ derivatisation were successfully performed using a mixture of solvent and derivatising agent. The protection of the moisture-sensitive derivatising agent was afforded by the hydrophobic hollow fibre. Parameters such as type of derivatising agent, extraction solvent, pH, salt concentration, stirring speed and extraction time were optimised using spiked deionised water samples. The linear range established was between 0.05 and 25 microg ml⁽⁻¹⁾ depending on analyte, with squared regression coefficients ranging from 0.9959 to 0.9996. Relative standard deviations (RSDs) ranged from 6% to 10%. As comparison, solid-phase microextraction (SPME) was also evaluated and extraction conditions such as pH, salt concentration, stirring speed and extraction time were optimised. This work also represented the first report of such an in-situ derivatisation approach for SPME of basic analytes. The linear range established was between 0.5 and 25 microg ml⁽⁻¹⁾ depending on analyte, with squared regression coefficients ranging from 0.9946 to 0.9998. RSDs ranged from 5% to 22%."

"The limits of detection of HF-LPME (0.04-0.36 microg I(-1)) showed improvement over those of SPME (0.06-0.77 microg I(-1))."

The full article can be found at: Journal of Chromatography A (H.S. Lee, et. al., "Determination of basic degradation products of chemical warfare agents in water using hollow fibre-protected liquid-phase microextraction with in-situ derivatisation followed by gas chromatography-mass spectrometry". Journal of Chromatography A, 2008;1196-1197 (125-32). Link not available.

[Return to Top](#)

END of CB Daily Report.

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

Sandy Banks: Sandra.Banks@anser.org

Steve Tesko: Steve.Tesko@anser.org

Heather Williams: Heather.Williams@anser.org

Suzanne Martinez: Suzanne.Martinez@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.