

17 September 2009

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## **Chem-Bio News – S&T Edition**

- 1. US ARMY BOTULINUM NEUROTOXIN (BoNT) MEDICAL THERAPEUTICS RESEARCH PROGRAM: PAST ACCOMPLISHMENTS AND FUTURE DIRECTIONS:** *"Efforts have focused on molecules to inhibit nearly every aspect of BoNT pathogenesis."*
- 2. ENHANCING THE ELECTROCHEMICAL RESPONSE OF MYOGLOBIN WITH CARBON NANOTUBE ELECTRODES:** *"These findings encourage the use of myoglobin-modified carbon nanotube electrodes as potential (bio) sensors of H<sub>2</sub>O<sub>2</sub> or O<sub>2</sub> in biology, microbiology and environmental fields.."*
- 3. CAPTURING SINGLE MOLECULES OF IMMUNOGLOBULIN AND RICIN WITH AN APTAMER-ENCODED GLASS NANOPORE:** *"These findings will facilitate the development of a. universal nanopore for multitarget detection.."*
- 4. THE INHIBITION OF THE INTERACTION BETWEEN THE ANTHRAX TOXIN AND ITS CELLULAR RECEPTOR BY AN ANTI-RECEPTOR MONOCLONAL ANTIBODY:** *"To our knowledge, this is the first report to illustrate that an anti-CMG2 antibody Could inhibit the PA-CMG2 interaction and therefore interfere with the intoxication of anthrax toxin."*
- 5. SUBSTRATE RECOGNITION OF ANTHRAX LETHAL FACTOR EXAMINED BY COMBINATORIAL AND PRE-STEADY-STATE KINETIC APPROACHES:** *"Based on the available structural and kinetic data, we propose a model for LF-substrate interaction."*
- 6. DIFFERENTIAL MODULATION OF NF-KAPPA B-MEDIATED PRO-INFLAMMATORY RESPONSE IN HUMAN INTESTINAL EPITHELIAL CELLS BY CHEY HOMOLOGUES OF VIBRIO CHOLERA:** *"Further, the absence of nuclear translocation of NF-kappa B p50 subunit upon infection with O395Y3N or O395Y4N and its reversal upon complementation indicates the involvement of cheY-3 and cheY-4 in V. cholerae-induced pro-inflammatory response in the INT407 cell line.."*
- 7. GENETIC DETERMINATION OF ESSENTIAL RESIDUES OF THE VIBRIO CHOLERAEE ACTIN CROSS-LINKING DOMAIN REVEALS FUNCTIONAL SIMILARITY WITH GLUTAMINE SYNTHETASES:** *"Thus, the ACDs are a family of bacterial toxin effectors that may be evolutionarily related to ligases involved in amino acid biosynthesis."*
- 8. GR1+ CELLS CONTROL GROWTH OF YOPM-NEGATIVE YERSINIA PESTIS DURING SYSTEMIC PLAGUE:** *"Infection with fully virulent Y. pestis CO92 and a YopM(-) derivative by intradermal and intranasal routes showed that the absence of YopM significantly increased the 50% lethal dose only in the intradermal model, suggesting a role for YopM in bubonic plague, in which acute inflammation occurs soon after infection."*
- 9. GENE EXPRESSION PROFILING OF YERSINIA PESTIS WITH DELETION OF LCRG, A KNOWN NEGATIVE REGULATOR FOR YOP SECRETION OF TYPE III SECRETION SYSTEM:** *"Furthermore, this report also revealed significant transcriptional changes in the genes encoding cell-envelope-related proteins and a virulence-related transcription factor RovA in the Delta IcrG mutant."*
- 10. NEW RESEARCH CONFIRMS POTENTIAL DEADLY NATURE OF EMERGING NEW MONKEY MALARIA SPECIES IN HUMANS:** *"Researchers in Malaysia have identified key laboratory and clinical features of an emerging new form of malaria infection. The research, funded by the Wellcome Trust, confirms the potentially deadly nature of the disease."*
- 11. NEW ADJUVANT COULD HOLD THE FUTURE OF VACCINE DEVELOPMENT:** *"The new adjuvant is based on nanoparticles prepared with lecithin, a common food product. In animal models, it*

helped protein antigens to induce an immune response more than six times stronger than when alum was used."

**12. NEW 'ON-OFF' MEMBRANE FOR DRUG DELIVERY:** "US researchers have developed a new type of membrane that can be made reversibly porous at the flick of a switch."

## CB Daily Report

### Chem-Bio News

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#### **US ARMY BOTULINUM NEUROTOXIN (BoNT) MEDICAL THERAPEUTICS RESEARCH PROGRAM: PAST ACCOMPLISHMENTS AND FUTURE DIRECTIONS**

Drug Week

September 11, 2009

"The United States Army (USA) under the auspices of the Medical Research and Material Command (USAMRMC) and the Defense Threat Reduction Agency (DTRA) sponsored several major efforts to develop an effective medical countermeasure against botulinum neurotoxin (BoNT). This review focuses on the U.S.."

"Army's research and development efforts for a BoNT therapeutic over the period from 1975-2007. Two antitoxin preparations: Human botulism immunoglobulin (BIG) and Botulism Immune Globulin F(ab')<sub>2</sub> Heptavalent Equine (BIGHE) were administered to humans and shown to possess acceptable efficacy and safety levels. BIGHE was deployed in Operation Desert Storm/Desert Shield. BoNT/A monoclonal antibodies were developed and are currently undergoing clinical evaluation with funding from the National Institute of Allergy and Infectious Disease (NIAID). The development of small molecules for the treatment of BoNT has also been supported. Efforts have focused on molecules to inhibit nearly every aspect of BoNT pathogenesis. This would include toxin binding, translocation, catalytic activity, and recovery following intoxication. Several compounds capable of inhibiting toxin activity or mitigating the severity of paralysis have been identified. To date, none of the compounds possess the appropriate properties (safety, efficacy, solubility) to be considered for clinical studies."

The full article can be found at: (US Army Botulinum Neurotoxin (BoNT) Medical Therapeutics Research Program: Past Accomplishments and Future Directions. Drug Development Research, 2009;70(4 Sp. Iss.):266-278). Link not available.

[Return to Top](#)

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#### **ENHANCING THE ELECTROCHEMICAL RESPONSE OF MYOGLOBIN WITH CARBON NANOTUBE ELECTRODES**

Science Letter

September 8, 2009

"In particular, the performance of voltammetric biosensors made of forest-like carbon nanotubes, carbon nanotube composites and graphite composites is compared by monitoring mainly the electrocatalytic reduction of H<sub>2</sub>O<sub>2</sub> by myoglobin and their corresponding electroanalytical characteristics."

"Graphite composites showed the worst electroanalytical performance, exhibiting a small linear range, a limit of detection (LOD) of  $9 \times 10^{-5}$  M and low sensitivity. However, it was found that the electrochemical response was enhanced with the use of carbon nanotube-based electrodes with LOD up to  $5 \times 10^{-8}$  M, higher sensitivities and wider linear range response. On the one hand, in the case of the CNT epoxy composite, the improvement in the response can be mainly attributed to its more porous surface which allows the immobilization of higher amounts of the electroactive protein. On the other

hand, in the case of the forest-like CNT electrodes, the enhancement is due to an increase in the electron transfer kinetics."

"These findings encourage the use of myoglobin-modified carbon nanotube electrodes as potential (bio) sensors of H<sub>2</sub>O<sub>2</sub> or O<sub>2</sub> in biology, microbiology and environmental fields.."

The full article can be found at: (M.J. Esplandiu, et. al., "Enhancing the electrochemical response of myoglobin with carbon nanotube electrodes". Nanotechnology, 2009;20(35):55502). Link not available.

[Return to Top](#)

---

### **CAPTURING SINGLE MOLECULES OF IMMUNOGLOBULIN AND RICIN WITH AN APTAMER-ENCODED GLASS NANOPORE**

Chemical & Chemistry

September 18, 2009

"Synthetic nanopores are more stable and provide flexible pore sizes, but the selectivity is low when detecting in the translocation mode. In spite of modifications with probing molecules, such as antibodies, to potentiate. specific targeting, these nanopores fail to bind individual target molecules. Distinguishing between binding and translocation blocks remains unsolved. Here, we propose an aptamer-encoded nanopore that overcomes these challenges. Aptamers are well-known probing oligonucleotides that have high sensitivity and selectivity. In contrast to antibodies, aptamers are much smaller than their targets, rendering target: blockades in the nanopore much more distinguishable. We used aptamer-encoded nanopores to detect single molecules of immunoglobulin E and the bioterrorist agent ricin, sequentially captured by the immobilized aptamer in the sensing zone of the pore. The functional nanopore also probed sequence-dependent aptamer-protein interactions."

"These findings will facilitate the development of a. universal nanopore for multitarget detection.."

The full article can be found at: (S. Ding, et. al., "Capturing Single Molecules of Immunoglobulin and Ricin with an Aptamer-Encoded Glass Nanopore". Analytical Chemistry, 2009;81(16):6649-6655). Link not available.

[Return to Top](#)

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### **THE INHIBITION OF THE INTERACTION BETWEEN THE ANTHRAX TOXIN AND ITS CELLULAR RECEPTOR BY AN ANTI-RECEPTOR MONOCLONAL ANTIBODY**

Medical Letter on the CDC & FDA

September 6, 2009

"We demonstrated that one of the MAbs, 4B5, Could inhibit PA-CMG2 binding and could also protect the sensitive cells against an anthrax lethal toxin challenge. We identified the epitope recognized by 4B5 and confirmed that the key residues of the epitope were the residues (YI)-Y-119-LK125 of CMG2. Based on our results, we propose that 4B5 binds to the E122 pocket of CMG2 and interrupts the interaction between the pocket and the PA 2 beta 3-2 beta 4 loop."

"To our knowledge, this is the first report to illustrate that an anti-CMG2 antibody could inhibit the PA-CMG2 interaction and therefore interfere with the intoxication of anthrax toxin."

The full article can be found at: (G.L. Li, et. al., "The inhibition of the interaction between the anthrax toxin and its cellular receptor by an anti-receptor monoclonal antibody". Biochemical and Biophysical Research Communications, 2009;385(4):591-595). Link not available.

[Return to Top](#)

---

## **SUBSTRATE RECOGNITION OF ANTHRAX LETHAL FACTOR EXAMINED BY COMBINATORIAL AND PRE-STEADY-STATE KINETIC APPROACHES**

Medical Letter on the CDC & FDA

September 13, 2009

"In particular, new insights into the LF catalytic mechanism will be useful for the development of LF inhibitors. We evaluated the minimal length required for formation of bona fide LF substrates using substrate phage display. Phage-based selection yielded a substrate that is cleaved seven times more efficiently by LF than the peptide targeted in the protein kinase MKK6. Site-directed mutagenesis within the metal-binding site in the LF active center and within phage-selected substrates revealed a complex pattern of LF-substrate interactions. The elementary steps of LF-mediated proteolysis were resolved by the stopped-flow technique. Pre-steady-state kinetics of LF proteolysis followed a four-step mechanism as follows: initial substrate binding, rearrangement of the enzyme-substrate complex, a rate-limiting cleavage step, and product release. Examination of LF interactions with metal ions revealed an unexpected activation of the protease by Ca<sup>2+</sup> and Mn<sup>2+</sup>. Based on the available structural and kinetic data, we propose a model for LF-substrate interaction."

The full article can be found at: (M.Y. Zakharova, et. al., "Substrate Recognition of Anthrax Lethal Factor Examined by Combinatorial and Pre-steady-state Kinetic Approaches". Journal of Biological Chemistry, 2009;284(27):17902-17913). Link not available.

[Return to Top](#)

---

## **DIFFERENTIAL MODULATION OF NF-KAPPA B-MEDIATED PRO-INFLAMMATORY RESPONSE IN HUMAN INTESTINAL EPITHELIAL CELLS BY CHEY HOMOLOGUES OF VIBRIO CHOLERA**

Gastroenterology Week

September 14, 2009

"Vibrio cholerae, the etiological agent of cholera, colonizes the small intestine, produces an enterotoxin and causes acute inflammatory response at intestinal epithelial surface. Chemotaxis and motility greatly influence the infectivity of V. cholerae although the role of chemotaxis genes in V. cholerae pathogenesis is less well understood."

"Four cheY genes are present in three clusters in the complete genome sequence of V. cholerae. A less motile and less adherent mutant was generated by inactivation of cheY-3 (O395Y3N) or cheY-4 (O395Y4N) whereas alterations in motility or adherence were not observed for cheY-1 (O395Y1N) or cheY-2 (O395Y2N) insertional mutants. In contrast to O395Y1N and O395Y2N, O395Y3N and O395Y4N showed reduced cholera toxin production compared to wild-type in vitro. Infection of the human intestinal epithelial cell line Int407 with O395Y3N and O395Y4N caused reduced secretion of interleukin (IL)-1 alpha, IL-6, tumor necrosis factor (TNF-alpha) and monocyte chemoattractant protein-1 (MCP-1) compared to wild-type and was associated with delayed activation of nuclear factor kappa B (NF-kappa B) p65 and its co-activator cAMP response element binding protein (CREB)."

"Further, the absence of nuclear translocation of NF-kappa B p50 subunit upon infection with O395Y3N or O395Y4N and its reversal upon complementation indicates the involvement of cheY-3 and cheY-4 in V. cholerae-induced pro-inflammatory response in the INT407 cell line."

The full article can be found at: (A. Bandyopadhyaya, et. al., "Differential modulation of NF-kappa B-mediated pro-inflammatory response in human intestinal epithelial cells by cheY homologues of Vibrio cholera". Innate Immunity, 2009;15(3):131-142). Link not available.

[Return to Top](#)

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## **GENETIC DETERMINATION OF ESSENTIAL RESIDUES OF THE VIBRIO CHOLERAEE ACTIN CROSS-LINKING DOMAIN REVEALS FUNCTIONAL SIMILARITY WITH GLUTAMINE SYNTHETASES**

Drug Week

September 11, 2009

"Actin cross-linking domains (ACDs) are distinct domains found in several bacterial toxins, including the *Vibrio cholerae* MARTX toxin. The ACD of *V. cholerae* (ACD(Vc)) catalyses the formation of an irreversible iso-peptide bond between lysine 50 and glutamic acid 270 on two actin molecules in an ATP-and Mg/Mn(2+)-dependent manner."

"In vivo, cross-linking depletes the cellular pool of G-actin leading to actin cytoskeleton depolymerization. While the actin cross-linking reaction performed by these effector domains has been significantly characterized, the ACD(Vc) catalytic site has remained elusive due to lack of significant homology to known proteins. Using multiple genetic approaches, we have identified regions and amino acids of ACD(Vc) required for full actin cross-linking activity. Then, using these functional data and structural homology predictions, it was determined that several residues demonstrated to be important for ACD(Vc) activity are conserved with active-site residues of the glutamine synthetase family of enzymes."

"Thus, the ACDs are a family of bacterial toxin effectors that may be evolutionarily related to ligases involved in amino acid biosynthesis."

The full article can be found at: (B. Geissler, et. al., "Genetic determination of essential residues of the *Vibrio cholerae* actin cross-linking domain reveals functional similarity with glutamine synthetases". *Molecular Microbiology*, 2009;73(5):858-68). Link not available.

[Return to Top](#)

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## **GR1+ CELLS CONTROL GROWTH OF YOPM-NEGATIVE YERSINIA PESTIS DURING SYSTEMIC PLAGUE**

Medical Letter on the CDC & FDA

September 6, 2009

YopM, a protein toxin of *Yersinia pestis*, is necessary for virulence in a mouse model of systemic plague. We previously reported YopM-dependent natural killer (NK) cell depletion from blood and spleen samples of infected mice."

"However, in this study we found that infection with *Y. pestis* KIM5 (YopM(+)) caused depletion of NK cells in the spleen, but not in the liver, and antibody-mediated ablation of NK cells had no effect on bacterial growth. There was no YopM-associated effect on the percentage of dendritic cells (DCs) or polymorphonuclear leukocytes (PMNs) in the early stage of infection; however, there was a YopM-associated effect on PMN integrity and on the influx of monocytes into the spleen. Ablation of Gr1(+) cells caused loss of the growth defect of YopM(-) *Y. pestis* in both the liver and spleen. In contrast, ablation of macrophages/DCs inhibited growth of both parent and mutant bacteria, accompanied by significantly fewer lesion sites in the liver. These results point toward PMNs and inflammatory monocytes as major cell types that control growth of YopM(-) *Y. pestis*."

"Infection with fully virulent *Y. pestis* CO92 and a YopM(-) derivative by intradermal and intranasal routes showed that the absence of YopM significantly increased the 50% lethal dose only in the intradermal model, suggesting a role for YopM in bubonic plague, in which acute inflammation occurs soon after infection."

The full article can be found at: (Z. Ye, et. al., "Gr1+ cells control growth of YopM-negative yersinia pestis during systemic plague". *Infection and Immunity*, 2009;77(9):3791-806). Link not available.

[Return to Top](#)

---

## **GENE EXPRESSION PROFILING OF YERSINIA PESTIS WITH DELETION OF LCRG, A KNOWN NEGATIVE REGULATOR FOR YOP SECRETION OF TYPE III SECRETION SYSTEM**

Health Risk Factor Week

September 8, 2009

“To further understand the effect of lcrG deletion on Y pestis T3SS regulation, transcriptional profiles from the Delta lcrG mutant and wild-type Y pestis strains were compared. The results showed that although the Delta lcrG mutant was markedly attenuated (600-fold increase of LD50 in s.c. challenged BALB/c mice), transcriptions of almost all the type III genes were upregulated significantly in the Delta lcrG mutant. The immunoblotting analysis of YopM and LcrV demonstrated that their expressions were also increased in the Delta lcrG mutant in comparison to the wild-type strain. We speculate that, in addition to the negative regulation of the Yop secretion, LcrG could possibly play a negative regulatory role in the transcription of T3SS genes through indirect mechanisms.”

"Furthermore, this report also revealed significant transcriptional changes in the genes encoding cell-envelope-related proteins and a virulence-related transcription factor RovA in the Delta lcrG mutant."

The full article can be found at: (Z.M. Du, et. al., "Gene expression profiling of Yersinia pestis with deletion of lcrG, a known negative regulator for Yop secretion of type III secretion system". International Journal of Medical Microbiology, 2009;299(5):355-366). Link not available.

[Return to Top](#)

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## **NEW RESEARCH CONFIRMS POTENTIAL DEADLY NATURE OF EMERGING NEW MONKEY MALARIA SPECIES IN HUMANS**

Physorg.com

September 09, 2009

“Researchers in Malaysia have identified key laboratory and clinical features of an emerging new form of malaria infection. The research, funded by the Wellcome Trust, confirms the potentially deadly nature of the disease.”

“Recently, researchers at the University Malaysia Sarawak, led by Professors Balbir Singh and Janet Cox-Singh, showed that *P. knowlesi*, a malaria parasite previously thought to mainly infect only monkeys - in particular long-tailed and pig-tailed macaques found in the rainforests of Southeast Asia - was widespread amongst humans in Malaysia. Subsequent reports in neighbouring Southeast Asian countries have led to the recognition of *P. knowlesi* as the fifth cause of malaria in humans.

Now, in a study published in the journal *Clinical Infectious Diseases*, Professors Singh and Cox-Singh, together with colleagues from University Malaysia Sarawak, Kapit Hospital and the University of Western Australia, have published the first detailed prospective study of the clinical and laboratory features of human *P. knowlesi* infections.

"*P. knowlesi* malaria can easily be confused with *P. malariae* since these two parasites look similar by microscopy, but the latter causes a benign form of malaria," says Professor Singh. "In fact, because the *P. knowlesi* parasites reproduce every twenty four hours in the blood, the disease can be potentially fatal, so early diagnosis and appropriate treatment is essential. Understanding the most common features of the disease will be important in helping make this diagnosis and in planning appropriate clinical management."

The full article can be found at: <http://www.physorg.com/news171745925.html>

[Return to Top](#)

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## **NEW ADJUVANT COULD HOLD THE FUTURE OF VACCINE DEVELOPMENT**

Infection Control Today Magazine

September 14, 2009

“Scientists at Oregon State University have developed a new "adjuvant" that could allow the creation of

important new vaccines, possibly become a universal vaccine carrier and help medical experts tackle many diseases more effectively.

Adjuvants are substances that are not immunogenic themselves, but increase the immune response when used in combination with a vaccine.

However, due to concerns about safety and toxicity, there's only a single vaccine adjuvant – aluminum hydroxide, or alum – that has been approved for human use in the United States. It's found in such common vaccines as hepatitis B and tetanus. But even though widely used, alum is comparatively weak and will only work with certain diseases.

The new adjuvant is based on nanoparticles prepared with lecithin, a common food product. In animal models, it helped protein antigens to induce an immune response more than six times stronger than when alum was used. Researchers also showed that the lecithin nanoparticles were able to help induce a reasonable antibody response after only one shot, whereas it took at least two shots for the alum adjuvant to work.

Based on their studies, researchers believe the lecithin nanoparticles have wide potential applications and possibly a good safety profile. Their findings were just published in the *Journal of Controlled Release*, a professional journal in the field of pharmaceuticals, in work supported by the National Institute of Allergy and Infectious Diseases.”

The full article can be found at: <http://www.infectioncontroltoday.com/hotnews/new-adjuvant-vaccine-development.html>

[Return to Top](#)

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## **NEW 'ON-OFF' MEMBRANE FOR DRUG DELIVERY**

By Simon Hadlington

Chemistry World

September 14, 2009

“US researchers have developed a new type of membrane that can be made reversibly porous at the flick of a switch. A drug contained within a membrane-based implant could be released 'on demand' the scientists suggest, making it a potentially useful and novel way for the controlled delivery of drugs such as anaesthetics. Once the dose has been delivered, the membrane can be re-sealed until the next dose is required.

A team led by Daniel Kohane of Harvard Medical School in Boston, US, harnessed the thermosensitive properties of poly(N-isopropylacrylamide) (PNIPAM) to form the basis of the new system. This material can form a hydrogel which is swollen in its native state but which collapses upon heating.

The researchers embedded nanoscale particles of PNIPAM-based gels in an ethyl cellulose membrane so that clumps of the particles spanned the width of the membrane. They also entrapped magnetite nanoparticles within the membrane matrix.

If the membrane is exposed to an oscillating magnetic field, the magnetite nanoparticles heat up, in turn warming the PNIPAM by a few degrees - sufficient to cause the particles to collapse but not so high as to affect surrounding tissue. This leaves voids in the membrane, opening up channels from one side to the other.”

The full article can be found at:

<http://www.rsc.org/chemistryworld/News/2009/September/14090901.asp>

[Return to Top](#)

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**Steve Tesko:** [Steve.Tesko@anser.org](mailto:Steve.Tesko@anser.org)

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