

8 October 2009

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Chem-Bio News – S&T Edition

1. NOD1/NOD2-MEDIATED RECOGNITION PLAYS A CRITICAL ROLE IN INDUCTION OF ADAPTIVE IMMUNITY TO ANTHRAX AFTER AEROSOL EXPOSURE:

"We also identify a critical role for Nod1/Nod2 in priming responses after B. anthracis aerosol exposure, as mice deficient in Nod1/Nod2 were impaired in their ability to mount an anamnestic antibody response and were more susceptible to secondary lethal challenge than wild-type mice."

2. PAPER PESTICIDE SENSOR: *"Researchers have developed a paper-based colour-changing sensor to detect organophosphate pesticides and other acetylcholinesterase (AChE) inhibitors in food and drink samples."*

3. AN ASSAY FOR BOTULINUM TOXIN TYPES A, B AND F THAT REQUIRES BOTH FUNCTIONAL BINDING AND CATALYTIC ACTIVITIES WITHIN THE NEUROTOXIN:

"The EARB [endopeptidase activity, receptor-binding] assay system described is the first convenient in vitro assay system described which requires multiple functional biological activities with the BoNTs."

4. GENETIC CHARACTERIZATION OF CLOSTRIDIUM BOTULINUM ASSOCIATED WITH TYPE B INFANT BOTULISM IN JAPAN:

"We established a multiplex PCR assay for BoNT/B subtyping which will be useful for epidemiological studies of type B strains and the infectious diseases that they cause."

5. HOW T-CELL-DEPENDENT AND -INDEPENDENT CHALLENGES ACCESS THE BRAIN: VASCULAR AND NEURAL RESPONSES TO BACTERIAL

LIPOPOLYSACCHARIDE AND STAPHYLOCOCCAL ENTEROTOXIN B: *"Despite partial overlap in their neuronal and vascular response profiles, LPS and SEB appear to use distinct mechanisms to access the brain."*

6. STATISTICAL APPROACH TO ESTIMATE VACCINIA-SPECIFIC NEUTRALIZING ANTIBODY TITERS USING A HIGH-THROUGHPUT ASSAY:

"Here we describe the adaptation of a beta-galactosidase reporter-based vaccinia virus neutralization assay to large-scale use in a study that included over 1,000 subjects. We also describe the statistical methods involved in analyzing the large quantity of data generated."

CB Daily Report

Chem-Bio News

NOD1/NOD2-MEDIATED RECOGNITION PLAYS A CRITICAL ROLE IN INDUCTION OF ADAPTIVE IMMUNITY TO ANTHRAX AFTER AEROSOL EXPOSURE

Medical Letter on the CDC & FDA

October 11, 2009

"Toll-like receptors and Nod-like receptors (NLR) play an important role in sensing invading microorganisms for pathogen clearance and eliciting adaptive immunity for protection against rechallenge. Nod1 and Nod2, members of the NLR family, are capable of detecting bacterial peptidoglycan motifs in the host cytosol for triggering proinflammatory cytokine production."

"In the current study, we sought to determine if Nod1/Nod2 are involved in sensing *Bacillus anthracis* infection and eliciting protective immune responses. Using mice deficient in both Nod1 and Nod2 proteins, we showed that Nod1/Nod2 are involved in detecting *B. anthracis* for production of tumor necrosis factor alpha, interleukin-1 alpha (IL-1 alpha), IL-1 beta, CCL5, IL-6, and KC. Proinflammatory responses were higher when cells were exposed to viable spores than when they were exposed to irradiated spores, indicating that recognition of vegetative bacilli through Nod1/Nod2 is significant."

"We also identify a critical role for Nod1/Nod2 in priming responses after *B. anthracis* aerosol exposure, as mice deficient in Nod1/Nod2 were impaired in their ability to mount an anamnestic antibody response and were more susceptible to secondary lethal challenge than wild-type mice."

The full article can be found at: (C.L. Loving, et. al., "Nod1/Nod2-mediated recognition plays a critical role in induction of adaptive immunity to anthrax after aerosol exposure". *Infection and Immunity*, 2009; 77(10):4529-37). Link not available.

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PAPER PESTICIDE SENSOR

By Rajendrani Mukhopadhyay

Chemistry World

October 05, 2009

"Researchers have developed a paper-based colour-changing sensor to detect organophosphate pesticides and other acetylcholinesterase (AChE) inhibitors in food and drink samples. John Brennan and colleagues at McMaster University in Canada have developed a simple reagentless device that detects nanomolar quantities of pesticides within five minutes in samples like milk and lettuce."

Paper is an appealing material for analytical devices because it is relatively cheap, abundant, and can move fluids by capillary action without external power. A recent focus on paper-based, colour change-based diagnostic platforms has emerged because they may be useful in locations where resources are limited.

Brennan's team used AChE as a reporter because it is inhibited by pesticides such as organophosphates and carbamates. 'Organophosphates are still used in the developing world for spraying agricultural crops,' explains Brennan. 'The AChE-based sensor has the potential to provide rapid testing for organophosphates in the field.'

Piezoelectric inkjet printing is used to deposit reagents on a paper-based support to prepare it for use, so that additional reagents aren't needed at the time of analysis. AChE and a colour-changing substrate, indophenyl acetate (IPA), were trapped in two separate zones on a 1 × 10 cm paper strip using biocompatible sol-gel derived silica inks, with the enzyme trapped in a sensing zone and the IPA held in a substrate zone.

To test a sample, the end of the paper sensor is placed in the sample solution, which flows via capillary action to the sensing zone where it is left to incubate. Then, the opposite end of the sensor is dipped into distilled deionised water so that flow in the opposite direction pushes the IPA from the substrate zone to the sensing area. There, the AChE hydrolyses the IPA, creating a colour change from yellow to blue. The intensity of the blue colour observed (either by the naked eye or with a digital camera) is inversely proportional to the amount of pesticide in the sample. Brennan says the bidirectional flow approach significantly improves the detection limits because it lets the analytes incubate in the AChE-sensing zone before the IPA appears."

The full article can be found at: <http://www.rsc.org/chemistryworld/News/2009/October/05100901.asp>

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AN ASSAY FOR BOTULINUM TOXIN TYPES A, B AND F THAT REQUIRES BOTH FUNCTIONAL BINDING AND CATALYTIC ACTIVITIES WITHIN THE NEUROTOXIN

Drug Week

October 9, 2009

"To develop a novel assay technique for the botulinum neurotoxin family (BoNTs) which is dependent on both the endopeptidase and receptor-binding activities of the BoNTs and which is insensitive to antigenic variation with the toxin family. An endopeptidase activity, receptor-binding assay (EARB assay) has been developed which captures biologically active toxin from media using brain synaptosomes."

"After capture, the bound toxin can be incubated with its substrate, and cleavage detected using serotype-specific antibodies raised against the cleaved product of each toxin serotype. The EARB assay was assessed using a range of BoNT serotypes and subtypes. For BoNT/A, detection limits for subtypes A(1), A(2) and A(3) were 0.5, 3 and 10 MLD(50) ml(-1),

respectively. The limit of detection for BoNT/B(1) was 5 MLD(50) ml(-1) and a novel antibody-based endopeptidase assay for BoNT/F detected toxin at 0.5 MLD(50) ml(-1). All these BoNTs can be captured from media containing up to 10% serum without loss of sensitivity. BoNT/A(1) could also be detected in dilutions of a lactose-containing formulation similar to that used for clinical preparations of the toxin. Different serotypes were found to possess different optimal cleavage pHs (pH 6.5 for A(1), pH 7.4 for B(1)). The EARB assay has been shown to be able to detect a broad range of BoNT serotypes and subtypes from various media. AND IMPACT OF THE STUDY: The EARB assay system described is the first convenient in vitro assay system described which requires multiple functional biological activities with the BoNTs."

The full article can be found at: (E.R. Evans, et. al., "An assay for botulinum toxin types A, B and F that requires both functional binding and catalytic activities within the neurotoxin". Journal of Applied Microbiology, 2009; 107(4): 1384-91). Link not available.

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GENETIC CHARACTERIZATION OF CLOSTRIDIUM BOTULINUM ASSOCIATED WITH TYPE B INFANT BOTULISM IN JAPAN

TB & Outbreaks Week

October 13, 2009

"The 15 proteolytic *Clostridium botulinum* type B strains, including 3 isolates associated with infant botulism in Japan, were genetically characterized by phylogenetic analysis of boNT/ B gene sequences, genotyping, and determination of the boNT/ B gene location by using pulsed-field gel electrophoresis (PFGE) for molecular epidemiological analysis of infant botulism in Japan. Strain Osaka05, isolated from a case in 2005, showed a unique boNT/ B gene sequence and was considered to be a new BoNT/B subtype by phylogenetic analysis."

"Strain Osaka06, isolated from a case in 2006, was classified as the B2 subtype, the same as strain 111, isolated from a case in 1995. The five isolates associated with infant botulism in the United States were classified into the B1 subtype. Isolates from food samples in Japan were divided into the B1 and the B2 subtypes, although no relation with infant botulism was shown by PFGE genotyping. The results of PFGE and Southern blot hybridization with undigested DNA suggested that the boNT/ B gene is located on large plasmids (approximately 150 kbp, 260 kbp, 275 kbp, or 280 kbp) in five strains belonging to three BoNT/B subtypes from various sources. The botulinum neurotoxin (BoNT) of Osaka05 was suggested to have an antigenicity different from the antigenicities of BoNT/B1 and BoNT/B2 by a sandwich enzyme-linked immunosorbent assay with the recombinant BoNT/B-C-terminal domain."

"We established a multiplex PCR assay for BoNT/B subtyping which will be useful for epidemiological studies of type B strains and the infectious diseases that they cause."

The full article can be found at: (K. Umeda, et. al., "Genetic Characterization of *Clostridium botulinum* Associated with Type B Infant Botulism in Japan". Journal of Clinical Microbiology, 2009; 47(9): 2720-2728). Link not available.

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HOW T-CELL-DEPENDENT AND -INDEPENDENT CHALLENGES ACCESS THE BRAIN: VASCULAR AND NEURAL RESPONSES TO BACTERIAL LIPOPOLYSACCHARIDE AND STAPHYLOCOCCAL ENTEROTOXIN B

Biotech Week
October 7, 2009

"Bacterial lipopolysaccharide (LPS) is widely used to study immune influences on the CNS, and cerebrovascular prostaglandin (PG) synthesis is implicated in mediating LPS influences on some acute phase responses. Other bacterial products, such as staphylococcal enterotoxin B (SEB), impact target tissues differently in that their effects are T-lymphocyte-dependent, yet both LPS and SEB recruit a partially overlapping set of subcortical central autonomic cell groups."

"We sought to compare neurovascular responses to the two pathogens, and the mechanisms by which they may access the brain. Rats received iv injections of LPS (2 microg/kg), SEB (1mg/kg) or vehicle and were sacrificed 0.5-3h later. Both challenges engaged vascular cells as early 0.5h, as evidenced by induced expression of the vascular early response gene (Verge), and the immediate-early gene, NGFI-B. Cyclooxygenase-2 (COX-2) expression was detected in both endothelial and perivascular cells (PVCs) in response to LPS, but only in PVCs of SEB-challenged animals. The non-selective COX inhibitor, indomethacin (1mg/kg, iv), blocked LPS-induced activation in a subset of central autonomic structures, but failed to alter SEB-driven responses. Liposome mediated ablation of PVCs modulated the CNS response to LPS, did not affect the SEB-induced activational profile. By contrast, disruptions of interoceptive signaling by area postrema lesions or vagotomy (complete or hepatic) markedly attenuated SEB-, but not LPS-, stimulated central activational responses."

"Despite partial overlap in their neuronal and vascular response profiles, LPS and SEB appear to use distinct mechanisms to access the brain."

The full article can be found at: (J. Serrats, et. al., "How T-cell-dependent and -independent challenges access the brain: vascular and neural responses to bacterial lipopolysaccharide and staphylococcal enterotoxin B". *Brain, Behavior, and Immunity*, 2009; 23(7): 1038-52). Link not available.

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STATISTICAL APPROACH TO ESTIMATE VACCINIA-SPECIFIC NEUTRALIZING ANTIBODY TITERS USING A HIGH-THROUGHPUT ASSAY

Medical Letter on the CDC & FDA
October 11, 2009

"Here we describe the adaptation of a beta-galactosidase reporter-based vaccinia virus

neutralization assay to large-scale use in a study that included over 1,000 subjects. We also describe the statistical methods involved in analyzing the large quantity of data generated."

"The assay and its associated methods should prove useful tools in monitoring immune responses to next-generation smallpox vaccines, studying poxvirus immunity, and evaluating therapeutic agents such as vaccinia virus immune globulin."

The full article can be found at: (R. Kennedy, et. al., "Statistical Approach To Estimate Vaccinia-Specific Neutralizing Antibody Titers Using a High-Throughput Assay". *Clinical and Vaccine Immunology*, 2009; 16(8): 1105-1112). Link not available.

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Steve Tesko: Steve.Tesko@anser.org

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