

20 August 2009

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### **Chem-Bio News – S&T Edition**

#### **1. INTRANASAL IMMUNIZATION WITH RECOMBINANT TOXIN-COREGULATED PILUS AND CHOLERA TOXIN B SUBUNIT PROTECTS RABBITS AGAINST VIBRIO CHOLERAЕ O1**

**CHALLENGE:** *"Thus, mucosal codelivery of pertinent cholera toxoids provides enhanced protection against experimental cholera."*

#### **2. INHIBITION OF VACCINIA VIRUS ENTRY BY A BROAD SPECTRUM ANTIVIRAL PEPTIDE:**

*"The EB peptide is, to our knowledge, the first known small molecule inhibitor of Vaccinia virus entry."*

#### **3. SURFACE PLASMON RESONANCE-BASED IMMUNOSENSOR WITH ORIENTED IMMOBILIZED ANTIBODY FRAGMENTS ON A MIXED SELF-ASSEMBLED MONOLAYER FOR THE**

**DETERMINATION OF STAPHYLOCOCCAL ENTEROTOXIN B:** *"Furthermore, the sensor can be regenerated using 0.1 M HCl, and 70% of the initial response was maintained over 3 cycles.."*

#### **4. RAXIBACUMAB FOR THE TREATMENT OF INHALATIONAL ANTHRAX:**

*"A single dose of raxibacumab improved survival in rabbits and monkeys with symptomatic inhalational anthrax."*

#### **5. EVIDENCE FOR A PROTON-PROTEIN SYMPORT MECHANISM IN THE ANTHRAX TOXIN**

**CHANNEL:** *"We also find that a site that disfavors the entry of negatively charged residues into the (PA(63))(7) channel resides at or near its Phi-clamp, the ring of seven phenylalanines near the channel's entrance.."*

#### **6. CDC WANTS CLOSER CONTROLS ON RODENT-BORNE SOUTH AMERICAN CHAPARE VIRUS:**

*"The Centers for Disease Control and Prevention (CDC) is proposing to add the Chapare virus, which has caused the death of at least one man in Bolivia, to its list of select agents and toxins that can only be possessed by U.S. laboratories meeting certain safety and security standards."*

## **CB Daily Report**

### **Chem-Bio News**

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#### **INTRANASAL IMMUNIZATION WITH RECOMBINANT TOXIN-COREGULATED PILUS AND CHOLERA TOXIN B SUBUNIT PROTECTS RABBITS AGAINST VIBRIO CHOLERAЕ O1 CHALLENGE**

Immunotherapy Weekly  
August 12, 2009

"Intranasal immunization, a noninvasive method of vaccination, has been found to be effective in inducing systemic and mucosal immune responses. The present study was aimed at investigating the efficacy of intranasal immunization in inducing mucosal immunity in experimental cholera by subunit recombinant protein vaccines from *Vibrio cholerae* O1."

"The structural genes encoding toxin-coregulated pilus A (TcpA) and B subunit of cholera toxin (CtxB) from *V. cholerae* O1 were cloned and expressed in *Escherichia coli*. Rabbits were immunized intranasally with purified TcpA and CtxB alone or a mixture of TcpA and CtxB. Immunization with TcpA and CtxB alone conferred, respectively, 41.1% and 70.5% protection against *V. cholerae* challenge,

whereas immunization with a mixture of both antigens conferred complete (100%) protection, as assayed in the rabbit ileal loop model. Serum titers of immunoglobulin G (IgG) antibodies to TcpA and CtxB, and anti-TcpA- and anti-CtxB-specific sIgA in intestinal lavage of vaccinated animals were found to be significantly elevated compared with unimmunized controls. Vibriocidal antibodies were detected at remarkable levels in rabbits receiving TcpA antigen and their titers correlated with protection."

"Thus, mucosal codelivery of pertinent cholera toxoids provides enhanced protection against experimental cholera."

The full article can be found at: (J. Kundu, et. al., "Intranasal immunization with recombinant toxin-coregulated pilus and cholera toxin B subunit protects rabbits against *Vibrio cholerae* O1 challenge". *Fems Immunology and Medical Microbiology*, 2009;56(2):179-184). Link not available.

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## **INHIBITION OF VACCINIA VIRUS ENTRY BY A BROAD SPECTRUM ANTIVIRAL PEPTIDE**

Medical Imaging Week

August 15, 2009

"We identified a peptide, EB, which inhibited infection by Vaccinia virus with an EC50 of 15 mc M. A control peptide, EBX, identical in composition to EB but differing in sequence, was inactive (EC50 >200 mc M), indicating sequence specificity."

"The inhibition was reversed upon removal of the peptide, and EB treatment had no effect on the physical integrity of virus particles as determined by electron microscopy. Viral adsorption was unaffected by the presence of EB, and the addition of EB post-entry had no effect on viral titers or on early gene expression. The addition of EB post-adsorption resulted in the inhibition of beta-galactosidase expression from an early viral Promoter with an EC50 of 45 mc M. A significant reduction in virus entry was detected in the presence of the peptide when the number of viral cores released into the cytoplasm was quantified. Electron microscopy indicated that 88% of the virions remained on the surface of cells in the presence of EB, compared to 37% in the control (p <0.001). EB also blocked fusion-from-within, suggesting that virus infection is inhibited at the fusion step. Analysis of EB derivatives suggested that peptide length may be important for the activity of EB."

"The EB peptide is, to our knowledge, the first known small molecule inhibitor of Vaccinia virus entry."

The full article can be found at: (S.E. Altman, et. al., "Inhibition of Vaccinia virus entry by a broad spectrum antiviral peptide". *Virology*, 2009;388(2):248-259). Link not available.

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## **SURFACE PLASMON RESONANCE-BASED IMMUNOSENSOR WITH ORIENTED IMMOBILIZED ANTIBODY FRAGMENTS ON A MIXED SELF-ASSEMBLED MONOLAYER FOR THE DETERMINATION OF STAPHYLOCOCCAL ENTEROTOXIN B**

China Weekly News

August 18, 2009

"An immunosensor based on surface plasmon resonance (SPR) with a mixed self-assembled monolayer (SAM) was developed to determine staphylococcal enterotoxin B (SEB). The SAM on a gold surface was fabricated by adsorbing a mixture of 16-mercapto-1-hexadecanoic acid (16-MHA) and hexanethiol at various molar ratios."

"Initially, full-length anti-SEB was randomly immobilized onto the SAM to form the immunosensing surface. Through optimization of surface functionalization and anti-SEB immobilization, the SPR sensors can be applied to the determination of SEB in a linear range of 0.01 similar to 1.0  $\mu\text{g mL}^{-1}$ . Furthermore, a smaller antibody fragment (F(ab)') was generated and immobilized randomly (via amino groups) or in an oriented manner (via -SH groups) to form the immunosensing surface. The oriented

immobilization of F(ab)' led to a 50% increase in the antigen binding efficiency compared to randomly immobilized covalent F(ab') fragments. The resulting calibration curve showed higher sensitivity. In addition, the specificity and applicability of the proposed immunosensor to milk samples were also demonstrated."

"Furthermore, the sensor can be regenerated using 0.1 M HCl, and 70% of the initial response was maintained over 3 cycles.."

The full article can be found at: (W.C.Tsai, et. al., "Surface plasmon resonance-based immunosensor with oriented immobilized antibody fragments on a mixed self-assembled monolayer for the determination of staphylococcal enterotoxin B". *Microchimica Acta*, 2009;166(1-2):115-122). Link not available.

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## **RAXIBACUMAB FOR THE TREATMENT OF INHALATIONAL ANTHRAX**

Medical Letter on the CDC & FDA

August 16, 2009

"We evaluated the efficacy of raxibacumab as a prophylactic agent and after disease onset in a total of four randomized, placebo-controlled studies conducted in rabbits and monkeys. Animals were exposed to an aerosolized target exposure of *B. anthracis* spores that was approximately 100 times (in the prophylactic studies) and 200 times (in the therapeutic-intervention studies) the median lethal dose. In the therapeutic-intervention studies, animals were monitored for the onset of symptoms. Animals with detectable protective antigen in serum, a significant increase in temperature, or both received a single intravenous bolus of placebo or raxibacumab at a dose of either 20 mg per kilogram of body weight or 40 mg per kilogram. The primary end point was survival at day 14 (in rabbits) or at day 28 (in monkeys). Safety studies were conducted with intravenous raxibacumab (40 mg per kilogram) in 333 healthy human volunteers. In both rabbits and monkeys, the time to detection of protective antigen correlated with the time to bacteremia ( $r=0.9$ ,  $P<0.001$ ). In the therapeutic-intervention studies, the survival rate was significantly higher among rabbits that received raxibacumab at a dose of 40 mg per kilogram (44% [8 of 18]) than among rabbits that received placebo (0% [0 of 18];  $P=0.003$ ). Raxibacumab treatment also significantly increased survival in monkeys (64% [9 of 14], vs. 0% [0 of 12] with placebo;  $P<0.001$ ). In human subjects, intravenous raxibacumab at a dose of 40 mg per kilogram had a half-life of 20 to 22 days and provided a maximum concentration of the drug in excess of levels that are protective in animals. Concentrations of raxibacumab provide a surrogate end point that should be predictive of clinical benefit."

"A single dose of raxibacumab improved survival in rabbits and monkeys with symptomatic inhalational anthrax."

The full article can be found at: (T.S. Migone, et. al., "Raxibacumab for the Treatment of Inhalational Anthrax". *New U.K. Journal of Medicine*, 2009;361(2):135-144). Link not available.

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## **EVIDENCE FOR A PROTON-PROTEIN SYMPORT MECHANISM IN THE ANTHRAX TOXIN CHANNEL**

Health & Medicine Week

August 10, 2009

"Protein translocation through the channel is driven by a proton electrochemical potential gradient on a time scale of seconds. A paradoxical aspect of this is that although LFN (the N-terminal 263 residues of LF), on which most of our experiments were performed, has a net negative charge, it is driven through the channel by a cis-positive voltage. We have explained this by claiming that the (PA(63))(7) channel strongly disfavors the entry of negatively charged residues on proteins to be translocated, and hence the aspartates and glutamates on LFN enter protonated (i.e., neutralized). Therefore, the translocated

species is positively charged. Upon exiting the channel, the protons that were picked up from the cis solution are released into the trans solution, thereby making this a proton-protein symporter. Here, we provide further evidence of such a mechanism by showing that if only one SO<sub>3</sub><sup>-</sup>, which is essentially not titratable, is introduced at most positions in LFN, through the reaction of an introduced cysteine residue at those positions with 2-sulfonato-ethyl-methanethiosulfonate, voltage-driven LFN translocation is drastically inhibited."

"We also find that a site that disfavors the entry of negatively charged residues into the (PA(63))(7) channel resides at or near its Phi-clamp, the ring of seven phenylalanines near the channel's entrance.."

The full article can be found at: (D. Basilio, et. al., "Evidence for a Proton-Protein Symport Mechanism in the Anthrax Toxin Channel". Journal of General Physiology, 2009;133(3):307-314). Link not available.

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## **CDC WANTS CLOSER CONTROLS ON RODENT-BORNE SOUTH AMERICAN CHAPARE VIRUS**

By Jacob Goodwin

Government Security News

August 19, 2009

"The Centers for Disease Control and Prevention (CDC) is proposing to add the Chapare virus, which has caused the death of at least one man in Bolivia, to its list of select agents and toxins that can only be possessed by U.S. laboratories meeting certain safety and security standards."

.....

"We are proposing this action because Chapare virus has been phylogenetically identified as a Clade B arenavirus and is closely related to other currently regulated South American arenaviruses that cause haemorrhagic fever, particularly Sabia virus," said the CDC in a notice of proposed rulemaking it published in the Federal Register on August 19."

The full article can be found at: <http://www.gsnmagazine.com/cms/features/news-analysis/2535.html>

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