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CB Daily Report

Chem-Bio News

PROTECTION AGAINST ANTHRAX AND PLAGUE BY A COMBINED VACCINE IN MICE AND RABBITS

Medical Letter on the CDC & FDA
February 14, 2010

"The protective antigen (PA) of *Bacillus anthracis* and the Fraction 1 Capsular Antigen (F1 antigen), V antigen of *Yersinia pestis* have been demonstrated to be potential immunogens and candidate vaccine sub-units against anthrax and plague respectively. In this study, the authors have investigated the antibody responses and the protective efficacy when the antigens were administered separately or in combination intramuscularly formulation adsorbed to an aluminum hydroxide adjuvant."

"Immunized rF1 + rV and rPA antigen together was as effective as separately for induction of serological antibody response, and these titers were maintained for over 1 year in mice. An isotype analysis of the serum indicates that the co-administration of these antigens did not influence the antigen-specific IgG1/IgG2a ratio which was consistent with a Th2 bias. Furthermore, the combined vaccine comprising the protein antigens rF1 + rV + rPA has been demonstrated to protect mice from subcutaneous challenge with 10(7) colony-forming units (CFU) virulent *Y. pestis* strain, and to fully protect rabbit against subcutaneous challenge with 1.2 x 10(5) colony-forming units (CFU) virulent *B. anthracis* spores."

"These data show that the protective efficacy was unaffected when the antigens were administered in combination."

The full article can be found at: (J. Ren, et. al., "Protection against anthrax and plague by a combined vaccine in mice and rabbits". *Vaccine*, 2009;27(52):7436-7441). Link not available.

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CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS-ENCODED OVARIAN TUMOR PROTEASE ACTIVITY IS DISPENSABLE FOR VIRUS RNA POLYMERASE FUNCTION

Medical Letter on the CDC & FDA

February 14, 2010

"Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus (genus *Nairovirus*, family *Bunyaviridae*) associated with high case fatality disease outbreaks in regions of Africa, Europe, and Asia. The CCHFV genome consists of three negative-strand RNA segments, S, M, and L. The unusually large virus L polymerase protein and the need for biosafety level 4 (BSL-4) containment conditions for work with infectious virus have hampered the study of CCHFV replication."

"The L protein has an ovarian tumor (OTU) protease domain located in the N terminus, which has led to speculation that the protein may be autoproteolytically cleaved to generate the active virus L polymerase and additional functions. We report the successful development of efficient CCHFV helper virus-independent S, M, and L segment minigenome systems for analysis of virus RNA and protein features involved in replication. The virus RNA segment S, M, and L untranslated regions were found to be similar in support of replication of the respective minigenomes. In addition, the OTU domain located in the N terminus of the expressed virus L protein was shown to be a functional protease. However, no evidence of L protein autoproteolytic processing was found, and the OTU protease activity was dispensable for virus RNA replication. Finally, physiologically relevant doses of ribavirin inhibited CCHFV minigenome replication."

"These results demonstrated the utility of the minigenome system for use in BSL-2 laboratory settings to analyze CCHFV biology and in antiviral drug discovery programs for this important public health and bioterrorism threat."

The full article can be found at: (E. Bergeron, et. al., "Crimean-Congo Hemorrhagic Fever Virus-Encoded Ovarian Tumor Protease Activity Is Dispensable for Virus RNA Polymerase Function". *Journal of Virology*, 2010;84(1):216-226). Link not available.

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CONJUGATIVE TRANSFER OF INSECTICIDAL PLASMID PHT73 FROM BACILLUS THURINGIENSIS TO B. ANTHRACIS AND COMPATIBILITY OF THIS PLASMID WITH PXO1 AND

PX02

Medical Letter on the CDC & FDA
February 14, 2010

"Bacillus anthracis, the etiologic agent of anthrax, is genetically close to and commonly shares a giant gene pool with *B. cereus* and *B. thuringiensis*. In view of the human pathogenicity and the long persistence in the environment of *B. anthracis*, there is growing concern about the effects of genetic exchange with *B. anthracis* on public health."

"In this work, we demonstrate that an insecticidal plasmid, pHT73, from *B. thuringiensis* strain KT0 could be efficiently transferred into two attenuated *B. anthracis* strains, Ba63002R (pXO1(+) pXO2(-)) and Ba63605R (pXO1(-) pXO2(+)), by conjugation in liquid medium in the laboratory, with transfer rates of 2.3×10^{-4} and 1.6×10^{-4} CFU/donor, respectively. The *B. anthracis* transconjugants containing both pHT73 and pXO1 or pXO2 could produce crystal protein Cry1Ac encoded by plasmid pHT73 and had high toxicity to *Helicoverpa armigera* larvae. Furthermore, the compatibility and stability of pHT73 with pXO1/pXO2 were demonstrated."

"The data are informative for further investigation of the safety of *B. thuringiensis* and closely related strains in food and in the environment."

The full article can be found at: (Y.M. Yuan, et. al., "Conjugative Transfer of Insecticidal Plasmid pHT73 from *Bacillus thuringiensis* to *B. anthracis* and Compatibility of This Plasmid with pXO1 and pXO2". *Applied and Environmental Microbiology*, 2010;76(2):468-473). Link not available.

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SURFACE PLASMON RESONANCE ANALYSIS OF RICIN BINDING TO PLASMA MEMBRANES ISOLATED FROM NIH 3T3 CELLS

Medical Letter on the CDC & FDA
February 14, 2010

"In this work, surface plasmon resonance (SPR) was used to monitor binding of ricin, a ribosome-inactivating protein, to the plasma membranes of NIH 3T3 cells."

"Once conditions were established, efficacy of the system for monitoring effectiveness of compounds at inhibiting ricin binding was ascertained by determining the IC50 values for asialofetuin (ASF) and for bovine serum albumin derivatized with an average of 34 lactosyl moieties (BSA-Lac(34)). indicated that SPR is an efficient method for measuring adherence of a toxin to isolated cell plasma membranes."

"SPR can also indicate whether a compound that is an effective inhibitor of binding when a single ligand such as ASF is used will be as effective when used in studies with cells that may express multiple cell surface ligands for ricin and/or the inhibitor."

The full article can be found at: (M.C. Blome, et. al., "Surface plasmon resonance analysis of ricin binding to plasma membranes isolated from NIH 3T3 cells". *Analytical Biochemistry*, 2010;396(2):212-216). Link not available.

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REGULATORY INTERACTIONS OF A VIRULENCE-ASSOCIATED SERINE/THREONINE PHOSPHATASE-KINASE PAIR IN BACILLUS ANTHRACIS

Medical Letter on the CDC & FDA
February 14, 2010

"In the current study, we examined the regulatory interactions of a serine/threonine phosphatase (BA-Stp1), serine/threonine kinase (BA-Stk1) pair in *Bacillus anthracis*. *B. anthracis* STPK101, a null mutant lacking BA-Stp1 and BA-Stk1, was impaired in its ability to survive within macrophages, and this

correlated with an observed reduction in virulence in a mouse model of pulmonary anthrax. Biochemical analyses confirmed that BA-Stp1 is a PP2C phosphatase and dephosphorylates phosphoserine and phosphothreonine residues.”

“Treatment of BA-Stk1 with BA-Stp1 altered BA-Stk1 kinase activity, indicating that the enzymatic function of BA-Stk1 can be influenced by BA-Stp1 dephosphorylation. Using a combination of mass spectrometry and mutagenesis approaches, three phosphorylated residues, T165, S173, and S214, in BA-Stk1 were identified as putative regulatory targets of BA-Stp1. Further analysis found that T165 and S173 were necessary for optimal substrate phosphorylation, while S214 was necessary for complete ATP hydrolysis, autophosphorylation, and substrate phosphorylation.”

“These findings provide insight into a previously undescribed Stp/Stk pair in *B. anthracis*.”

The full article can be found at: (S.M. Shakit, et. al., “Regulatory interactions of a virulence-associated serine/threonine phosphatase-kinase pair in *Bacillus anthracis*”. *Journal of Bacteriology*, 2010;192(2):400-9). Link not available.

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CAPTURING OF CELL CULTURE-DERIVED MODIFIED VACCINIA ANKARA VIRUS BY ION EXCHANGE AND PSEUDO-AFFINITY MEMBRANE ADSORBERS [MA]

By M.W.Wolfe, et. al.

Biotechnology & Bioengineering

March 2010

“However, the threat of using smallpox as a biological weapon prompted efforts of some governments to produce smallpox vaccines for emergency preparedness. An additional aspect for the interest in smallpox virus is its potential use as a platform technology for vector vaccines. In particular, the latter requires a high safety level for routine applications. IMVAMUNE, a third generation smallpox vaccine based on the attenuated Modified Vaccinia Ankara (MVA) virus, demonstrates superior safety compared to earlier generations and represents therefore an interesting choice as viral vector. Current downstream production processes of Vaccinia virus and MVA are mainly based on labor-intensive centrifugation and filtration methods, requiring expensive nuclease treatment in order to achieve sufficient low host-cell DNA levels for human vaccines. This study compares different ion exchange and pseudo-affinity membrane adsorbers (MA) to capture chicken embryo fibroblast cell-derived MVA-BN after cell homogenization and clarification. In parallel, the overall performance of classical bead-based resin chromatography (Cellufine sulfate and Toyopearl AF-Heparin) was investigated. The two tested pseudo-affinity MA (i.e., sulfated cellulose and heparin) were superior over the applied ion exchange MA in terms of virus yield and contaminant depletion. Furthermore, studies confirmed an expected increase in productivity resulting from the increased volume throughput of MA compared to classical bead-based column chromatography methods. Overall virus recovery was approximately 60% for both pseudo-affinity MA and the Cellufine sulfate resin. Depletion of total protein ranged between 86% and 102% for all tested matrices. Remaining dsDNA in the product fraction varied between 24% and 7% for the pseudo-affinity chromatography materials. Cellufine sulfate and the reinforced sulfated cellulose MA achieved the lowest dsDNA product contamination.”

“Finally, by a combination of pseudo-affinity with anion exchange MA a further reduction of host-cell DNA was achieved.”

The full article can be found at: (M.W. Wolff, et. al., “Capturing of cell culture-derived modified Vaccinia Ankara virus by ion exchange and pseudo-affinity membrane adsorbers [MA]”. *Biotechnology & Bioengineering*, 2010;105(4):761-9). Link not available.

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SCIENTISTS RAISE FRESH HOPES FOR FRIDGE-FREE VACCINES

By Jane Dreaper

BBC
February 18, 2010

“Scientists at Oxford University have found a way of keeping vaccines stable without refrigeration.

Writing in *Science Translational Medicine*, they say the breakthrough could significantly help efforts to immunise more children in rural Africa.

The researchers mixed the vaccines with two types of sugar before slowly drying them on a filter paper.

This preserved the jabs, which were then easily reactivated when needed for injection.”

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“Writing in the journal *Science Translational Medicine*, the scientists describe how they managed to keep vaccines stable for up to six months at 45C.

They used sucrose and another sugar called trehalose, which is known for its preservative properties.”

.....

“Our tests were pretty tough as we used live viruses. So we feel that having stabilised those more fragile vaccines, this method should work for other vaccines containing dead protein.”

The full article can be found at: <http://news.bbc.co.uk/2/hi/health/8520825.stm>

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SCIENTISTS DISCOVER HOW PROTEIN TRIPS UP BACTERIA

Infection Control Today Magazine
February 17, 2010

“Now, Johns Hopkins scientists have shown that a healthy immune response depends on a protein called TRPV2 which, they discovered, is the means by which macrophages capitalize on brief and accidental encounters with nasty bugs.

Reporting in *Nature Immunology* in the Jan. 31 online edition, the team proves that TRPV2 is necessary not only for macrophages to get a good grip on disease-causing bacteria, but also as the first line of defense, rallying the rest of the immune system to dispose of the most slippery and sizable germs.”

The full article can be found at: <http://www.infectioncontrolday.com/hotnews/protein-trips-up-bacteria.html>

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