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Chem-Bio News – S&T Edition

1. FIMH ADHESIN OF TYPE 1 FIMBRIAE IS A POTENT INDUCER OF INNATE ANTIMICROBIAL RESPONSES WHICH REQUIRES TLR4 AND TYPE 1 INTERFERON

SIGNALLING: *"Our studies suggest that FimH can potentially be used as an innate microbicide against mucosal pathogens."*

2. COMBATING THE THREAT OF ANTHRAX: A QUANTITATIVE STRUCTURE-ACTIVITY

RELATIONSHIP APPROACH: *"Internal (cross-validation (q(2)), quality factor (Q), Fischer statistics (F), and Y-randomization) and external validation tests have validated all the QSAR [Quantitative structure-activity relationship] models."*

3. A SINGLE IMMUNIZATION WITH A DRY POWDER ANTHRAX VACCINE PROTECTS

RABBITS AGAINST LETHAL AEROSOL CHALLENGE: *"These data demonstrate that a single immunization with our dry powder anthrax vaccine can protect against a lethal aerosol spore challenge 9 weeks later."*

4. DEVELOPMENT OF ISOTHERMAL TAQMAN ASSAYS FOR DETECTION OF

BIOTHREAT ORGANISMS: *"With this platform, we have successfully developed rapid real-time isothermal assays for biodefense targets that include Vibrio cholerae and Bacillus anthracis."*

5. CONSTRUCTION OF A VIBRIO CHOLERAЕ PROTOTYPE VACCINE STRAIN O395-N1-E1 WHICH ACCUMULATES CELL-ASSOCIATED CHOLERA TOXIN B SUBUNIT:

"Our results suggest that this prototype cholera vaccine candidate strain may assist in preparing improved and inexpensive oral BS-WC cholera vaccine without the need to purify CTB separately"

6. DUAL-PROBE REAL-TIME PCR ASSAY FOR DETECTION OF VARIOLOA OR OTHER

ORTHOPOXVIRUSES WITH DRIED REAGENTS: *"Furthermore, the implementation of dried reagents in real-time PCR assays is an important step towards simplifying such assays and allowing their use in areas where cold storage is not easily accessible."*

CB Daily Report

FIMH ADHESIN OF TYPE 1 FIMBRIAE IS A POTENT INDUCER OF INNATE ANTIMICROBIAL RESPONSES WHICH REQUIRES TLR4 AND TYPE 1 INTERFERON SIGNALLING

By Ali A. Ashkar, Karen L. Mossman, Brian K. Coombes, Carlton L. Gyles, Randy Mackenzie
PLoS Pathogens

December 11, 2008

"Components of bacteria have been shown to induce innate antiviral immunity via Toll-like receptors (TLRs). We have recently shown that FimH, the adhesin portion of type 1 fimbria, can induce the innate immune system via TLR4. Here we report that FimH induces potent in vitro and in vivo innate antimicrobial responses. FimH induced an innate antiviral state in murine macrophage and primary MEFs which was correlated with IFN- β production. Moreover, FimH induced the innate antiviral responses in cells from wild type, but not from MyD88 $^{-/-}$, Trif $^{-/-}$, IFN- α/β R $^{-/-}$ or IRF3 $^{-/-}$ mice. Vaginal delivery of FimH, but not LPS, completely protected wild type, but not MyD88 $^{-/-}$, IFN- α/β R $^{-/-}$, IRF3 $^{-/-}$ or TLR4 $^{-/-}$ mice from subsequent genital HSV-2 challenge. The FimH-induced innate antiviral immunity correlated with the production of IFN- β , but not IFN- α or IFN- γ . To examine whether FimH plays a role in innate immune induction in the context of a natural infection, the innate immune responses to wild type uropathogenic E. coli (UPEC) and a FimH null mutant were examined in the urinary tract of C57Bl/6 (B6) mice and TLR4-deficient mice. While UPEC expressing FimH induced a robust polymorphonuclear response in B6, but not TLR4 $^{-/-}$ mice, mutant bacteria lacking FimH did not. In addition, the presence of TLR4 was essential for innate control of and protection against UPEC. Our results demonstrate that FimH is a potent inducer of innate antimicrobial responses and signals differently, from that of LPS, via TLR4 at mucosal surfaces. Our studies suggest that FimH can potentially be used as an innate microbicide against mucosal pathogens."

The full article can be found at: <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000233;jsessionid=3DC3998E6AEFA263B7D0478308C88778>

[Return to Top](#)

COMBATING THE THREAT OF ANTHRAX: A QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP APPROACH

Medical Letter on the CDC & FDA

December 21, 2008

"In this paper, we developed 7 QSAR [Quantitative structure-activity relationship] models on penicillin-based inhibitors of the class A and B beta-lactamases from B. anthracis and inhibitors of anthrax lethal factor to understand the chemical -biological interactions. Hydrophobic and steric factors are found to be the most important determinants of the activity."

"Internal (cross-validation (q(2)), quality factor (Q), Fischer statistics (F), and Y-randomization) and external validation tests have validated all the QSAR models."

The full article can be found at: (R.P. Verma, et. al., "Combating the threat of anthrax: A quantitative structure-activity relationship approach". *Molecular Pharmaceutics*, 2008;5 (5): 745-759. Link not available.

[Return to Top](#)

A SINGLE IMMUNIZATION WITH A DRY POWDER ANTHRAX VACCINE PROTECTS RABBITS AGAINST LETHAL AEROSOL CHALLENGE

Drug Week

December 19, 2008

"Here we confirm that intranasal (IN) dry powder anthrax vaccine formulations are able to protect rabbits against aerosol challenge 9 weeks after a single immunization. The optimum dose of rPA in our dry powder anthrax vaccine formulation in rabbits was experimentally determined to be 150microg and therefore was chosen as the target dose for all subsequent experiments."

"Rabbits received a single dose of either 150microg rPA, 150microg rPA+150microg of a conjugated 10-mer peptide representing the *Bacillus anthracis* capsule (conj), or 150microg of conj alone. All dry powder formulations contained MPL and chitosan (ChiSys). Significant anti-rPA titers and anthrax lethal toxin neutralizing antibody (TNA) levels were seen with both rPA containing vaccines, although rPA-specific IgG and TNA levels were reduced in rabbits immunized with rPA plus conj. Nine weeks after immunization, rabbits were exposed to a mean aerosol challenge dose of 278 LD50 of Ames spores. Groups immunized with rPA or with rPA+conj had significant increases in survivor proportions compared to the negative control group by Logrank test ($p=0.0001$ and 0.003 , respectively), and survival was not statistically different for the rPA and rPA+conj immunized groups ($p=0.63$)."

"These data demonstrate that a single immunization with our dry powder anthrax vaccine can protect against a lethal aerosol spore challenge 9 weeks later."

The full article can be found at: (S.D. Klas, et. al., "A single immunization with a dry powder anthrax vaccine protects rabbits against lethal aerosol challenge". *Vaccine*, 2008;26 (43):5494-502). Link not available.

[Return to Top](#)

DEVELOPMENT OF ISOTHERMAL TAQMAN ASSAYS FOR DETECTION OF BIOTHRREAT ORGANISMS

Medical Devices & Surgical Technology Week

December 21, 2008

"TaqMan probe (dual-labeled DNA probe)-based real-time detection, one of the most sensitive and specific fluorescent detection methods, has been widely utilized in conjunction with polymerase chain reaction (PCR). Helicase-dependent amplification (HDA) is an isothermal amplification technology that has a similar reaction scheme to PCR, but replaces thermocycling with a helicase capable of unwinding a DNA duplex."

"Here we describe a novel isothermal real-time detection method (HDA-TaqMan) that combines the advantages of both HDA and a TaqMan assay. In this assay, the reactions of DNA unwinding, primer annealing, polymerization, probe hybridization, and subsequent hydrolysis by the polymerase are coordinated and synchronized to perform at a single temperature. It not only provides a useful tool for real-time detection of HDA, but also provides an isothermal format for the TaqMan system."

"With this platform, we have successfully developed rapid real-time isothermal assays for biodefense targets that include *Vibrio cholerae* and *Bacillus anthracis*."

The full article can be found at: (Y. Tong, et. al., "Development of isothermal TaqMan assays for detection of biothreat organisms". *Biotechniques*, 2008; 45(5): 543-57). Link not available.

[Return to Top](#)

CONSTRUCTION OF A VIBRIO CHOLERAЕ PROTOTYPE VACCINE STRAIN O395-N1-E1 WHICH ACCUMULATES CELL-ASSOCIATED CHOLERA TOXIN B SUBUNIT

Drug Week

December 19, 2008

"Both WC [whole cell] and BS-WC [whole cell-recombinant B subunit vaccine] vaccines produced moderate levels of protection in field trials designed to evaluate their cholera efficacy. *V. cholerae* cells in these vaccines induce antibacterial immunity, and CTB contributes to the vaccine's efficacy presumably by stimulating production of anti-toxin neutralizing antibody. Although more effective than the WC vaccine, the BS-WC vaccine has not been adopted for manufacture by developing world countries primarily because the CTB component is difficult to manufacture and include in the vaccine in the doses needed to induce significant immune responses. We reasoned this was a technical problem that might be solved by engineering strains of *V. cholerae* that express cell-associated CTB that would co-purify with the bacterial cell fraction during the manufacture of WC vaccine. Here we report that construction of a *V. cholerae* O1 classical strain, O395-N1-E1, that has been engineered to accumulate CTB in the periplasmic fraction by disrupting the *epsE* gene of type II secretion pathway. O395-N1-E1 induces anti-CTB IgG and vibriocidal antibodies in mice immunized with two doses of formalin killed whole cells. Intraperitoneal immunization of mice with O395-N1-E1 induced a significantly higher anti-CTB antibody response compared to that of the parental strain, O395-N1."

"Our results suggest that this prototype cholera vaccine candidate strain may assist in preparing improved and inexpensive oral BS-WC cholera vaccine without the need to purify CTB separately."

The full article can be found at: (G.E.Rhie, et. al., "Construction of a Vibrio cholerae prototype vaccine strain O395-N1-E1 which accumulates cell-associated cholera toxin B subunit". Vaccine, 2008;26(43):5443-8). Link not available.

[Return to Top](#)

DUAL-PROBE REAL-TIME PCR ASSAY FOR DETECTION OF VARIOLA OR OTHER ORTHOPOXVIRUSES WITH DRIED REAGENTS

Virus Weekly

December 9, 2008

"A real-time, multiplexed polymerase chain reaction (PCR) assay based on dried PCR reagents was developed. Only variola virus could be specifically detected by a FAM (6-carboxyfluorescein)-labeled probe while camelpox, cowpox, monkeypox and vaccinia viruses could be detected by a TET (6-carboxytetramethylrhodamine)-labeled probe in a single PCR reaction."

"Approximately 25 copies of cloned variola virus DNA and 50 copies of genomic orthopoxviruses DNA could be detected with high reproducibility. The assay exhibited a dynamic range of seven orders of magnitude with a correlation coefficient value greater than 0.97. The sensitivity and specificity of the assay, as determined from 100 samples that contained nucleic acids from a multitude of bacterial and viral species were 96% and 98%, respectively. The limit of detection, sensitivity and specificity of the assay were comparable to standard real-time PCR assays with wet reagents. Employing a multiplexed format in this assay allows simultaneous discrimination of the variola virus from other closely related orthopoxviruses."

"Furthermore, the implementation of dried reagents in real-time PCR assays is an important step towards simplifying such assays and allowing their use in areas where cold storage is not easily accessible."

The full article can be found at: (M. Aitichou, et. al., "Dual-probe real-time PCR assay for detection of variola or other orthopoxviruses with dried reagents". Journal of Virological Methods, 2008;153(2):190-5). Link not available.

[Return to Top](#)

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