

2 October 2008

This supplement has been prepared to present scientific and technical news items that may be of more interest to technical personnel at RDT&E activities and the labs, or the medics rather than the broader readership of the basic CB Daily. Due to the nature of the material, the articles, if available online, are usually only available through subscription services thus making specific links generally unavailable. Thus, usually only the bibliographic citation is available for use by an activity's technical library.

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Chem-Bio News – S&T Supplement

1. ANTHRAX LETHAL TOXIN ENHANCES TNF [TUMOR NECROSIS FACTOR]-INDUCED ENDOTHELIAL VCAM-1 [VASCULAR CELL ADHESION MOLECULE-1] EXPRESSION VIA AN IFN REGULATORY FACTOR-1-DEPENDENT MECHANISM: *"Altering the activity of key transcription factors involved in host response to infection may be a critical mechanism by which LT contributes to anthrax pathogenesis."*

2. NORWALK VIRUS SHEDDING AFTER EXPERIMENTAL HUMAN INFECTION: *"The development of more sensitive methods to detect noroviruses has been associated with a corresponding increase in the duration of recognized virus shedding."*

3. LENTIGEN CORPORATION AND THE UNITED STATES ARMY AGREE TO CONTRACT: *"This contract is for a further phase of Lentigen Corporation's programs, which focus on protein expression and biodefense applications of lentiviral vectors. The research work is to be undertaken in collaboration with the U.S. Army Edgewood Chemical Biological Center (ECBC)."*

4. GROUNDBREAKING DISCOVERY MAY LEAD TO STRONGER ANTIBIOTICS: *"A new breakthrough by University of Virginia researchers provides physicians and patients a potential new approach toward the creation of less resistant and more effective antibiotics."*

5. CELL ADHESION PROMOTES EBOLA VIRUS ENVELOPE GLYCOPROTEIN-MEDIATED BINDING AND INFECTION: *"Furthermore, with 293F cells the acquisition of EboV RBD binding paralleled cell spreading and did not require new mRNA or protein synthesis."*

6. DIFFERENTIAL ANTIGEN REQUIREMENTS FOR PROTECTION AGAINST SYSTEMIC AND INTRANASAL VACCINIA VIRUS CHALLENGES IN MICE: *"These studies also suggest that rAd vectors warrant further assessment as candidate subunit smallpox vaccines."*

7. HIDDEN INFECTIONS CRUCIAL TO UNDERSTANDING, CONTROLLING DISEASE OUTBREAKS: *"Scientists and news organizations typically focus on the number of dead and gravely ill during epidemics, but research at the University of Michigan suggests that less dramatic, mild infections lurking in large numbers of people are the key to understanding*

cycles of at least one potentially fatal infectious disease: cholera."

8. LONG-TERM PERSISTENCE OF VIRULENT YERSINIA PESTIS IN SOIL: *"This study is a first step on which to base further investigations of a potential telluric reservoir for Y. pestis, which could represent an alternative mechanism for the maintenance of plague foci."*

9. CONFORMATIONAL STATES AND ASSOCIATION MECHANISM OF YERSINIA PESTIS CAF1 SUBUNITS: *"Using these data, an end-to-end model of the fiber, which well agrees with available experimental data, was also generated."*

CB Daily Report

Chem-Bio News

ANTHRAX LETHAL TOXIN ENHANCES TNF [TUMOR NECROSIS FACTOR]-INDUCED ENDOTHELIAL VCAM-1 [VASCULAR CELL ADHESION MOLECULE-1] EXPRESSION VIA AN IFN REGULATORY FACTOR-1-DEPENDENT MECHANISM

Medical Letter on the CDC & FDA

October 5, 2008

"Impaired host defenses and vascular dysfunction are hallmarks of the late, antibiotic-refractory stages of systemic anthrax infection. Anthrax lethal toxin (LT), a key virulence factor of Bacillus anthracis, was previously shown to enhance VCAM-1 expression on primary human endothelial cells suggesting a causative link between dysregulated adhesion molecule expression and the poor immune response and vasculitis associated with anthrax."

"In this study, we report that LT amplification of TNF-induced VCAM-1 expression is driven transcriptionally by the cooperative activation of NF-kappa B and IFN regulatory factor-1 (IRF-1). LT enhancement of NF-kappa B phosphorylation and nuclear translocation correlated temporally with a delayed reaccumulation of I kappa B alpha, while increased induction of IRF-1 was linked to STAT1 activation. LT failed to augment TNF-induced ICAM-1 or E-selectin expression, two adhesion molecules regulated by NF-kappa B, but not IRF-1. These results suggest that LT can differentially modulate NF-kappa B target genes and highlight the importance of IRF-1 in VCAM-1 enhancement."

"Altering the activity of key transcription factors involved in host response to infection may be a critical mechanism by which LT contributes to anthrax pathogenesis."

The full article can be found at: (J.M. Warfel, et. al., "Anthrax lethal toxin enhances TNF-induced endothelial VCAM-1 expression via an IFN regulatory factor-1-dependent mechanism". Journal of Immunology, 2008; 180(11):7516-7524). Link not available.

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NORWALK VIRUS SHEDDING AFTER EXPERIMENTAL HUMAN INFECTION

By Robert L. Atmar, Comments to Author Antone R. Opekun, Mark A. Gilger, Mary K. Estes,

“Abstract

Noroviruses are the most common cause of viral gastroenteritis in the United States. To determine the magnitude and duration of virus shedding in feces, we evaluated persons who had been experimentally infected with Norwalk virus. Of 16 persons, clinical gastroenteritis (watery diarrhea and/or vomiting) developed in 11; symptomatic illness lasted 1–2 days. Virus shedding was first detected by reverse transcription–PCR (RT-PCR) 18 hours after participant inoculation and lasted a median of 28 days after inoculation (range 13–56 days). The median peak amount of virus shedding was 95×10^9 (range $0.5\text{--}1,640 \times 10^9$) genomic copies/g feces as measured by quantitative RT-PCR. Virus shedding was first detected by antigen ELISA ≈ 33 hours (median 42 hours) after inoculation and lasted 10 days (median 7 days) after inoculation. Understanding of the relevance of prolonged fecal norovirus excretion must await the development of sensitive methods to measure virus infectivity.”

“Discussion

Noroviruses are estimated to cause 23 million cases of gastroenteritis in the United States each year and to be the most common cause of foodborne gastroenteritis (13). Relatively few data describe the quantity and duration of fecal norovirus shedding as determined by modern assays. In a human experimental Norwalk virus infection model, we found that Norwalk virus could be detected in fecal samples for a median of 4 weeks and for up to 8 weeks after virus inoculation and that peak virus titers were most commonly found in fecal samples collected after resolution of symptoms. The peak virus titers (median 95×10^9 copies/g feces) were higher than would be expected from electron microscopic studies (5,14). These observations help explain the epidemiologic observations of norovirus outbreaks linked to food handlers who had recovered from symptomatic infection (15) and in persons who had no gastroenteritis (16).

Only a few studies have used quantitative RT-PCRs to examine fecal viral load, and these studies have been primarily of GII norovirus strains. Chan et al. (17) described patients who shed $>10^{11}$ norovirus copies/g feces, whereas the peak fecal virus titer observed by Ozawa et al. (18) in symptomatic and asymptomatic food handlers was ≈ 10 -fold lower. Each of these studies was of persons with naturally acquired norovirus infection. However, the median peak viral load observed in our study (10^{11}) was much higher than the $10^7\text{--}10^8$ median viral loads reported in the prior studies (17,18). Lee et al. (19) noted higher viral loads in patients who had more prolonged symptoms (>4 days) associated with infection caused by GII.4 norovirus. Amar et al. (20) also reported viral loads to be higher in persons who had symptomatic gastroenteritis than in those who had been asymptomatic for at least 3 weeks. Our findings suggest that clinical gastroenteritis was associated with higher peak virus shedding and higher total virus shedding during the first 2 weeks after inoculation. Although we did not see an association of peak virus titer with symptom duration, the median duration of symptoms averaged only ≈ 1 day in our study. Potential reasons for the different results observed in other studies include the manner in which samples were collected (single samples vs. serial collection), the real-time assays used (generic assays designed to be broadly reactive vs. assay designed specifically for Norwalk virus detection),

virulence of the infecting strains, differences in the populations studied (e.g., age, immune status), and the small number of infected persons who did not have clinical gastroenteritis in our study.

The development of more sensitive methods to detect noroviruses has been associated with a corresponding increase in the duration of recognized virus shedding (1,8). For example, Rockx et al. (21) found norovirus in fecal samples for >3 weeks in ≈25% of infected persons, and Murata et al. (22) found norovirus in fecal samples for up to 6 weeks in infected infants. In contrast, at least half of the participants in our study still had Norwalk virus in fecal samples after 4 weeks and 2 had virus still present at 8 weeks; we cannot exclude the possibility that these 2 persons shed for a longer period. Determination of whether the virus is still infectious must await the development of more sensitive and reproducible methods for norovirus cultivation than are currently available (23)."

The full link can be found at: <http://www.cdc.gov/eid/content/14/10/1553.htm>

ANALYST NOTE:

Norovirus, also known as "Norwalk virus" or "Norwalk-like virus" is an extremely infectious and debilitating gastrointestinal disease that normally makes the news in a cruise ship context. While tainted food is usually the primary mode of transmission, contaminated surfaces especially those in the vicinity of the sick also pose significant hazards.

Although not considered a CBW agent and despite its holiday association, its military implications should not be overlooked. Early in the war, in 2002, there were a number of outbreaks at Allied bases in Afghanistan. These outbreaks not only affected troops but also, due to its high communicability, medical personnel as well. The outbreak at Bagram Airfield among British forces, for example, resulted in approximately five deaths and the aeromedical evacuation back to the UK of other servicemembers. Thus, the disease can assume significance from a force health protection standpoint.

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LENTIGEN CORPORATION AND THE UNITED STATES ARMY AGREE TO CONTRACT

MarketWatch

October 1, 2008

"Lentigen Corporation announced today that it has established a contract with the United States Army to extend the programs established in the initial agreement announced in 2006. This contract is for a further phase of Lentigen Corporation's programs, which focus on protein expression and biodefense applications of lentiviral vectors. The research work is to be undertaken in collaboration with the U.S. Army Edgewood Chemical Biological Center (ECBC)."

"We have been able to successfully use Lentiviral vector technology to take an existing hybridoma that poorly produces a highly valued monoclonal antibody targeted to the Anthrax bacillus and create a new cell line that produces the antibody at high levels. The

technology can therefore be used to salvage highly valued antibodies from hybridomas that are poor producers or are at risk. The technology has also been successfully used to produce difficult-to-express proteins at levels suitable for further development and potential commercialization," said Boro Dropulic, Founder and CSO of Lentigen."

The full article can be found at: <http://www.marketwatch.com/news/story/lentigen-corporation-united-states-army/story.aspx?guid={5713E5A4-1C72-4C3C-AB93-4F6CD6FE0F26}&dist=hppr>

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GROUNDBREAKING DISCOVERY MAY LEAD TO STRONGER ANTIBIOTICS

Infection Control Today Magazine

October 1, 2008

"The last decade has seen a dramatic decline in the effectiveness of antibiotics, resulting in a mounting public health crisis across the world. A new breakthrough by University of Virginia researchers provides physicians and patients a potential new approach toward the creation of less resistant and more effective antibiotics."

"What Bushweller, professor of molecular physiology and biological physics, and fellow researchers at the UVA Health System and Harvard Medical School have determined is the structure of a particular integral membrane enzyme, called DsbB -- one of the many proteins that reside in cell membranes. These so-called integral membrane proteins are important, because they account for roughly one-third of any genome in the human body and are the targets of more than half of all currently used drugs."

The full article can be found at: <http://www.infectioncontrolday.com/hotnews/stronger-antibiotics.html>

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CELL ADHESION PROMOTES EBOLA VIRUS ENVELOPE GLYCOPROTEIN-MEDIATED BINDING AND INFECTION

Blood Weekly

October 9, 2008

"Ebola virus infects a wide variety of adherent cell types, while nonadherent cells are found to be refractory," investigators in the United States report (see also Ebola Hemorrhagic Fever).

"To explore this correlation, we compared the ability of pairs of related adherent and nonadherent cells to bind a recombinant Ebola virus receptor binding domain (EboV RBD) and to be infected with Ebola virus glycoprotein (GP)-pseudotyped particles. Both human 293F and THP-1 cells can be propagated as adherent or nonadherent cultures, and in both

cases adherent cells were found to be significantly more susceptible to both EboV RBD binding and GP-pseudotyped virus infection than their nonadherent counterparts."

"Furthermore, with 293F cells the acquisition of EboV RBD binding paralleled cell spreading and did not require new mRNA or protein synthesis."

The full article can be found at: (D. Dube, et. al., "Cell adhesion promotes Ebola virus envelope glycoprotein-mediated binding and infection". Journal of Virology, 2008; 82 (14):7238-7242). Link not available.

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DIFFERENTIAL ANTIGEN REQUIREMENTS FOR PROTECTION AGAINST SYSTEMIC AND INTRANASAL VACCINIA VIRUS CHALLENGES IN MICE

Vaccine Weekly
October 8, 2008

"The development of a subunit vaccine for smallpox represents a potential strategy to avoid the safety concerns associated with replication-competent vaccinia virus. Preclinical studies to date with subunit smallpox vaccine candidates, however, have been limited by incomplete information regarding protective antigens and the requirement for multiple boost immunizations to afford protective immunity."

"Here we explore the protective efficacy of replication-incompetent, recombinant adenovirus serotype 35 (rAd35) vectors expressing the vaccinia virus intracellular mature virion (IMV) antigens A27L and L1R and extracellular enveloped virion (EEV) antigens A33R and B5R in a murine vaccinia virus challenge model. A single immunization with the rAd35-L1R vector effectively protected mice against a lethal systemic vaccinia virus challenge. The rAd35-L1R vector also proved more efficacious than the combination of four rAd35 vectors expressing A27L, L1R, A33R, and B5R. Moreover, serum containing L1R-specific neutralizing antibodies afforded postexposure prophylaxis after systemic vaccinia virus infection. In contrast, the combination of rAd35-L1R and rAd35-B5R vectors was required to protect mice against a lethal intranasal vaccinia virus challenge, suggesting that both IMV- and EEV-specific immune responses are important following intranasal infection. Taken together, these data demonstrate that different protective antigens are required based on the route of vaccinia virus challenge."

"These studies also suggest that rAd vectors warrant further assessment as candidate subunit smallpox vaccines."

The full article can be found at: (D.R. Kaufman, et. al., "Differential antigen requirements for protection against systemic and intranasal vaccinia virus challenges in mice". Journal of Virology, 2008; 82(14):6829-6837). Link not available.

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HIDDEN INFECTIONS CRUCIAL TO UNDERSTANDING, CONTROLLING DISEASE OUTBREAKS

Health & Medicine Week

October 6, 2008

"Scientists and news organizations typically focus on the number of dead and gravely ill during epidemics, but research at the University of Michigan suggests that less dramatic, mild infections lurking in large numbers of people are the key to understanding cycles of at least one potentially fatal infectious disease: cholera.

Using a model developed with new statistical methods, U-M researchers and their collaborators came up with results that challenge longstanding assumptions about the disease and strategies for preventing it.

Their findings appear in the Aug. 14 issue of the journal Nature."

"What we found was a real surprise," said King, who has joint appointments in the Department of Mathematics and the Center for the Study of Complex Systems. "Our analysis showed that the best explanation for the patterns seen in the data is that many more people are being exposed to the bacteria [cholera] than are getting serious infections or dying, and that individuals with mild infections are losing their immunity quite quickly, in a matter of weeks or months."

The model revealed that as an epidemic spreads, many people develop this short-term immunity. Once large numbers of people are immune, the epidemic comes to a halt. "But before the year is out, they're susceptible again," and the cycle starts all over, King said.

The quick waning of immunity found in this study contrasts with the widely-held belief---based only on studies of people with severe cholera, not on those with mild cases---that immunity to reinfection lasts at least three and possibly as long as ten years. The most effective cholera vaccines, by contrast, produce an immunity that lasts only a few months. The new model raises the possibility that current vaccines could be given at the beginning of cholera season to squelch an incipient epidemic.

Link not available.

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LONG-TERM PERSISTENCE OF VIRULENT YERSINIA PESTIS IN SOIL

Medical Letter on the CDC & FDA

October 5, 2008

"Sterilized soil inoculated with virulent *Y. pestis* biotype *Orientalis* was regularly sampled for 40 weeks in duplicate. Each sample was observed by acridine orange staining and immunofluorescence using an anti-*Y. pestis* polyclonal antibody, and DNA was extracted for PCR amplification and sequencing of the *Y. pestis* *ureD*, *caf1* and *pla* genes. All samples

were inoculated onto selective agar, and samples from soil that had been incubated for 10, 60, 165, 210 and 280 days were also inoculated into each of two BALB/c female mice. The mouse experiment was performed in triplicate. Non-inoculated, sterilized soil samples were used as negative controls. Micro-organisms fluorescing orange and detected by immunofluorescence were identified as *Y. pestis* in all samples. They were recovered in pure agar cultures for up to 30 weeks but thereafter were contaminated with *Pseudomonas* spp. Soil that had been inoculated with *Y. pestis* proved to be fully virulent in mice, which died with *Y. pestis* septicaemia and multiple organ involvement. Negative control mice showed no signs of disease. These data indicate that *Y. pestis* biotype *Orientalis* can remain viable and fully virulent after 40 weeks in soil."

"This study is a first step on which to base further investigations of a potential telluric reservoir for *Y. pestis*, which could represent an alternative mechanism for the maintenance of plague foci."

The full article can be found at: (S. Ayyadurai, et. al., "Long-term persistence of virulent *Yersinia pestis* in soil". *Microbiology*, 2008; 154(Pt 9):2865-71). Link not available.

ANALYST NOTE:

Readers may wish to review the following article on this subject which was reported in the 5 June 2008 edition of the CB Daily – S&T Supplement:

PERSISTENCE OF YERSINIA PESTIS IN SOIL UNDER NATURAL CONDITIONS

By Rebecca J. Eisen, Jeannine M. Petersen, Charles L. Higgins, David Wong, Craig E. Levy, Paul S. Mead, Martin E. Schriefer, Kevin S. Griffith, Kenneth L. Gage, and C. Ben Beard
Emerging Infectious Diseases – US Centers for Disease Control
June 2008

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CONFORMATIONAL STATES AND ASSOCIATION MECHANISM OF YERSINIA PESTIS CAF1 SUBUNITS

Gene Therapy Weekly
October 2, 2008

"Bacterial infectivity often relies on efficient attachment to the host cells through adhesive extensions. Unveiling the structural basis of the formation of these organelles is of paramount importance for both academic and applicative implications."

"Computational approaches may fruitfully complement experimental studies by providing information on specific conformational states whose characterization is difficult. Here, we report molecular dynamics characterizations of *Yersinia pestis* Caf1 subunit in its monomeric-unbound and dimeric states. Data on the monomeric form indicate that it is highly reactive and evolves toward compact states, which likely hamper subunit-subunit association. In line with recent experimental reports, this finding implies that chaperone release and subunit-

subunit association must be simultaneous. MD analysis on Caf1 dimer lead to the formation of a novel assembly endowed with a significant stability in the simulation timescale."

"Using these data, an end-to-end model of the fiber, which well agrees with available experimental data, was also generated."

The full article can be found at: (L. Vitagliano, et. al., "Conformational states and association mechanism of Yersinia pestis Caf1 subunits". Biochemical and Biophysical Research Communications, 2008;372(4):804-810). Link not available.

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