

30 April 2009

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## **Chem-Bio News – S&T Edition**

- 1. SUSPECTED H1N1 FLU CASE REPORTED AT CALIFORNIA MARINE BASE:** *"The Marine Corps commandant today confirmed a suspected case of what the U.S. Homeland Security Council now is calling H1N1 flu, but has been known as "swine flu," at the Marine Corps Air Ground Combat Center, Twentynine Palms, Calif."*
- 2. WHO RAISES PANDEMIC ALERT TO PHASE 5:** *"To confront the spreading swine influenza H1N1 virus, the head of the World Health Organization (WHO) today raised the agency's pandemic alert level to phase 5, one notch below a full-fledged influenza pandemic, signaling that it's time for all countries to prepare."*
- 3. SWINE FLU CONTAINER EXPLODES ON TRAIN:** *"Luckily the virus was not the mutated swine flu that has killed around 150 people in Mexico and that has already spread to parts of Europe."*
- 4. MEXICO SHUTS GOVERNMENT; PANDEMIC IMMINENT:** *"Mexico's president told citizens on Wednesday to stay home for a five-day partial shutdown of the economy, after the World Health Organization raised its alert level and said a swine flu pandemic was imminent."*
- 5. CUTTING EDGE: MUCOSAL APPLICATION OF A LYOPHILIZED VIRAL VECTOR VACCINE CONFERS SYSTEMIC AND PROTECTIVE IMMUNITY TOWARD INTRACELLULAR PATHOGENS:** *"These data clearly demonstrate the potency of a simple needle-free vaccination, combining the advantages of mucosal application with the stability and efficiency of lyophilized MVA [modified vaccinia virus Ankara]."*
- 6. BASAL EXPRESSION OF METALLOTHIONEIN SUPPRESSES BUTENOLIDE-INDUCED OXIDATIVE STRESS IN LIVER HOMOGENATES IN VITRO:** *"These findings implicated the antioxidant potency of basal expression of NIT in suppression of the oxidative stress of butenolide."*
- 7. SURFACE PLASMON RESONANCE DETECTION USING ANTIBODY-LINKED MAGNETIC NANOPARTICLES FOR ANALYTE CAPTURE, PURIFICATION, CONCENTRATION, AND SIGNAL AMPLIFICATION:** *"The surface plasmon resonance (SPR) detection signal from staphylococcal enterotoxin B (SEB) was dramatically increased when the IMBs [immunomagnetic beads] were used as detection amplifiers."*
- 8. THIOAMIDE HYDROXYPYRATHIONES SUPERSEDE AMIDE HYDROXYPYRATHIONES IN POTENCY AGAINST ANTHRAX LETHAL FACTOR:** *"A structure-activity relationship (SAR) of potential lethal factor inhibitors (LFI) is presented in which the zinc-binding group (ZBG), linker, and backbone moieties for a series of hydroxypyrrone-based compounds were systematically varied."*
- 9. MOLECULAR ANALYSIS OF THE INTERACTION OF ANTHRAX ADENYLYL CYCLASE TOXIN, EDEMA FACTOR, WITH 2'(3')-O-(N-(METHYL)ANTHRANILOYL)-SUBSTITUTED PURINE AND PYRIMIDINE NUCLEOTIDES:** *"Like mAC, EF accommodates both purine and pyrimidine nucleotides. The unique preference of EF for the base cytosine offers an excellent starting point for the development of potent and selective EF inhibitors."*
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activity in Gram-positive bacteria."

# CB Daily Report

## Chem-Bio News

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### **SUSPECTED H1N1 FLU CASE REPORTED AT CALIFORNIA MARINE BASE**

By Fred W. Baker, III

American Forces Press Service on DefenseLink.mil

April 29, 2009

"The Marine Corps commandant today confirmed a suspected case of what the U.S. Homeland Security Council now is calling H1N1 flu, but has been known as "swine flu," at the Marine Corps Air Ground Combat Center, Twentynine Palms, Calif.

A male Marine reported to a medical clinic on base over the weekend complaining of flu-like symptoms. Initial test results indicate the H1N1 virus, and the service is waiting for further results from testing at the Centers for Disease Control and Prevention in Atlanta, Marine Corps Gen. James T. Conway said.

The Marine, along with his roommate, are quarantined in the barracks, Conway said, adding that the roommate has shown no symptoms and the infected Marine is recovering.

"He's doing fine," the general said. "He's up and about. He said he feels pretty good. The doctors tell us that at this point, there appears to be no threat to him in terms of loss of life."

Doctors at the base also identified and restricted the activities of 37 other Marines who may have had contact with the infected Marine. The 37 are not officially quarantined, but are not allowed in public places such as unit formations and dining facilities.

The infected Marine was not given Tamiflu, an anti-viral drug used to prevent the flu, because he was past the point in his illness at which the medicine would have been effective, Conway said. All of the other Marines involved are taking Tamiflu.

The southern-California base is about 200 miles from the Mexican border, but the Marine had not visited Mexico, Conway said.

Marine doctors should receive the test results from the CDC in the next two days.

This case comes on the heels of two military family members in Texas, both teenage boys, with confirmed cases this month. Both boys have made full recoveries.

Defense Department officials say they are monitoring the outbreak closely, with a primary focus on protecting the military population.

Two prescription anti-viral drugs, Relenza and Tamiflu, already are standard stock at U.S. military treatment facilities, and larger quantities are stockpiled at several sites in the United States and overseas, officials said."

The full article can be found at: <http://www.defenselink.mil/news/newsarticle.aspx?id=54124>

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**WHO RAISES PANDEMIC ALERT TO PHASE 5**

By Robert Roos

CIDRAP News (Center for Infectious Disease Research & Policy - University of Minnesota)

April 29, 2009

“To confront the spreading swine influenza H1N1 virus, the head of the World Health Organization (WHO) today raised the agency's pandemic alert level to phase 5, one notch below a full-fledged influenza pandemic, signaling that it's time for all countries to prepare.

The move comes just 2 days after the WHO raised the alert from phase 3, where it had been for several years, to phase 4.

"Based on assessment of all available information, and following several expert consultations, I have decided to raise the current level of influenza pandemic alert from phase 4 to phase 5," WHO Director-General Dr. Margaret Chan said in a prepared statement she read at a press conference in Geneva."

“Officially, a phase 5 declaration means that a novel virus has caused sustained community outbreaks in two or more countries within one WHO region. In response to questions, Chan signaled that the spreading cases in Mexico and the United States meet that criterion. The official count of confirmed US cases today rose to 91, including 51 in New York City.

The criterion for phase 5 is that "first we have to demonstrate human-to-human transmission in a sustainable manner and community spread, and we've seen this definitely in Mexico, and as information has emerged from US authorities, we are also seeing that" in the United States, Chan said.

"When we see two countries in one region providing evidence to that effect, we are moving into phase 5," she added.

Phase 6, the peak phase of the WHO pandemic scale, is defined as the time when the virus has caused sustained community outbreaks in more than one WHO region. Dr. Keiji Fukuda, the WHO's assistant director-general for health security, suggested that phase 6 may not be far off.

"A pandemic means we have spread of this new virus in multiple countries and multiple regions," he said in response to the question whether a pandemic is actually under way. "If this continues, we expect it will be in other countries and other regions."

He also called phase 5 "a situation in which the likelihood [of a pandemic] is very high to inevitable."

The full article can be found at:

<http://www.cidrap.umn.edu/cidrap/content/influenza/swineflu/news/apr2909who.html>

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## **SWINE FLU CONTAINER EXPLODES ON TRAIN**

Der Spiegel Online International

April 28, 2009

“When a container holding swine flu exploded on a Swiss train on Monday, it could have led to a nightmare scenario. Luckily the virus was not the mutated swine flu that has killed around 150 people in Mexico and that has already spread to parts of Europe.”

“According to the police, a lab technician with the Swiss National Center for Influenza in Geneva had travelled to Zurich to collect eight ampoules, five of which were filled with the H1N1 swine flu virus. The samples were to be used to develop a test for swine flu infections.

The containers were hermetically sealed and cooled with dry ice. However, it seems the dry ice was not packed correctly and it melted during the journey. The gas coming from the containers then built up too much pressure and the ampoules exploded, as the train was pulling into a station.”

The full article can be found at: <http://www.spiegel.de/international/zeitgeist/0,1518,621598,00.html>  
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### **MEXICO SHUTS GOVERNMENT; PANDEMIC IMMINENT**

Fox News  
April 30, 2009

"Mexico's president told citizens on Wednesday to stay home for a five-day partial shutdown of the economy, after the World Health Organization raised its alert level and said a swine flu pandemic was imminent.

In his first televised address since the crisis erupted last week, President Felipe Calderon told Mexicans to stay home with their families. The country will suspend non-essential work and services, including some government ministries, from May 1-5.

"There is no safer place than your own home to avoid being infected with the flu virus," Calderon said.

Mexico is taking the drastic step after another 17 deaths were potentially linked to swine flu, bringing the total to as many as 176.

Essential services such as transport, supermarkets, trash collection and hospitals will remain open."

The full article can be found at: <http://www.foxnews.com/story/0,2933,518426,00.html>  
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### **CUTTING EDGE: MUCOSAL APPLICATION OF A LYOPHILIZED VIRAL VECTOR VACCINE CONFERS SYSTEMIC AND PROTECTIVE IMMUNITY TOWARD INTRACELLULAR PATHOGENS**

Vaccine Weekly  
April 29, 2009

"A major problem of current vaccines is storage stability, often requiring strict maintenance of cold chains. In the course of the eradication of smallpox, a freeze-dried vaccinia virus (Dryvax), which proved to be very stable, was used to overcome this limitation."

"However, Dryvax needs to be reconstituted before usage and is administered using a bifurcated needle, procedures that pose a number of additional health risks. We report in this study that a stable, lyophilized, modified vaccinia virus Ankara (MVA) vaccine can be directly applied to the nostrils of mice without previous reconstitution. This direct mucosal application induced systemic Ab and T cell responses comparable to those achieved by i.m. administration. Importantly, mucosal application of lyophilized MVA induced long-lasting protective immunity against lethal bacterial and viral challenges. These data clearly demonstrate the potency of a simple needle-free vaccination, combining the advantages of mucosal application with the stability and efficiency of lyophilized MVA."

The full article can be found at: (W. Kastenmuller, et. al., "Cutting Edge: Mucosal Application of a Lyophilized Viral Vector Vaccine Confers Systemic and Protective Immunity toward Intracellular Pathogens". *Journal of Immunology*, 2009;182(5):2573-2577). Link not available.  
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### **BASAL EXPRESSION OF METALLOTHIONEIN SUPPRESSES BUTENOLIDE-INDUCED OXIDATIVE STRESS IN LIVER HOMOGENATES IN VITRO**

Health Risk Factor Week  
April 21, 2009

"Butenolide (4-acetamido-4-hydroxy-2-butenic acid gamma-lactone) is a Fusarium mycotoxin which is frequently detected in foodstuffs and feedstuffs for human and animal consumption. It can evoke a broad spectrum of toxicities, thus posing a potential health risk to both humans and animals."

"Previous study showed that this mycotoxin produced a significant oxidative stress, and several antioxidants abated this effect. Metallothionein (MT) has been proposed as a potent antioxidant, therefore, this study attempts to determine whether endogenous expression of MT protects against butenolide-induced hepatic oxidative stress by using an in vitro incubation system of liver homogenates prepared from MT-I/II null (MT<sup>-/-</sup>) mice, and the corresponding wild type (MT<sup>+/+</sup>) mice. The results showed that butenolide elicited significant oxidative stress in both MT<sup>-/-</sup> mice and MT<sup>+/+</sup> mice; however, MT<sup>-/-</sup> mice were more sensitive than MT<sup>+/+</sup> mice to butenolide-induced hepatic oxidative stress, as evidenced by more production of thiobarbituric acid reactive substances and nitric oxide, and by more severe reductions of glutathione, superoxide dismutase and glutathione peroxidase in the liver homogenates of MT<sup>-/-</sup> mice than those of MT<sup>+/+</sup> mice."

"These findings implicated the antioxidant potency of basal expression of NIT in suppression of the oxidative stress of butenolide."

The full article can be found at: (H.Y. Yang, et. al., "Basal expression of metallothionein suppresses butenolide-induced oxidative stress in liver homogenates in vitro". *Toxicol*, 2009;53(2):246-253). Link not available.

ANALYST NOTE: The POC for this study is given as: S.Q. Peng, Academy Military Med Science, Institute Diseases Control & Prevention, Evaluation & Research Center Toxicology, 20 Dong Da St., Beijing 100071, People's Republic of China

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## **SURFACE PLASMON RESONANCE DETECTION USING ANTIBODY-LINKED MAGNETIC NANOPARTICLES FOR ANALYTE CAPTURE, PURIFICATION, CONCENTRATION, AND SIGNAL AMPLIFICATION**

Science Letter

April 21, 2009

"We have developed a method for rapid purification, concentration, and detection of target analytes from complex matrixes using antibody-coated superparamagnetic nanobeads (immunomagnetic beads, or IMBs). The surface plasmon resonance (SPR) detection signal from staphylococcal enterotoxin B (SEB) was dramatically increased when the IMBs were used as detection amplifiers. When SEB detection included a 10-fold concentration/purification IMB protocol, a substantial increase in detection sensitivity was observed. This procedure was used to successfully purify and concentrate SEB from serum and stool samples, then amplify the SPR detection signal. SEB at a concentration of 100 pg/mL was easily detected in both buffer and stool samples using this procedure. The IMB protocol also served to verify the analyte detection by using two different anti-SEB antibodies, mouse monoclonal antibodies attached to the magnetic nanobeads and rabbit polyclonal antibodies on the SPR sensor surface."

"Multiple detections of SEB in stool were performed using the same sensor surface by regenerating the sensor surfaces with a pH 2.2 buffer wash."

The full article can be found at: (S.D. Soelberg, et. al., " Surface Plasmon Resonance Detection Using Antibody-Linked Magnetic Nanoparticles for Analyte Capture, Purification, Concentration, and Signal Amplification". *Analytical Chemistry*, 2009;81(6):2357-2363). Link not available.

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## **THIOAMIDE HYDROXYPYROTHIONES SUPERSEDE AMIDE HYDROXYPYROTHIONES IN POTENCY AGAINST ANTHRAX LETHAL FACTOR**

Health & Medicine Week  
April 20, 2009

"Anthrax lethal factor (LF) is a critical virulence factor in the pathogenesis of anthrax. A structure-activity relationship (SAR) of potential lethal factor inhibitors (LFI) is presented in which the zinc-binding group (ZBG), linker, and backbone moieties for a series of hydroxypyrrone-based compounds were systematically varied."

"It was found that hydroxypyrothione ZBGs generate more potent inhibitors than hydroxypyrrone ZBGs. Furthermore, coupling the hydroxypyrothione to a backbone group via a thioamide bond improves potency when compared to an amide linker. QM/MM studies show that the thioamide bond in these inhibitors allows for the formation of two additional hydrogen bonds with the protein active site. In both types of hydroxypyrothione compounds, ligand efficiencies of 0.29-0.54 kcal mol<sup>-1</sup> per heavy atom were achieved."

The full article can be found at: (Thioamide Hydroxypyrothiones Supersede Amide Hydroxypyrothiones in Potency against Anthrax Lethal Factor. *Journal of Medicinal Chemistry*, 2009;52(4):1063-1074).  
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### **MOLECULAR ANALYSIS OF THE INTERACTION OF ANTHRAX ADENYLYL CYCLASE TOXIN, EDEMA FACTOR, WITH 2'(3')-O-(N-(METHYL)ANTHRANILOYL)-SUBSTITUTED PURINE AND PYRIMIDINE NUCLEOTIDES**

Drug Week  
May 1, 2009

Previous studies from our laboratory showed that mammalian membranous AC (mAC) exhibits broad specificity for purine and pyrimidine nucleotides (*Mol Pharmacol* 70:878-886, 2006). Here, we investigated structural requirements for EF inhibition by natural purine and pyrimidine nucleotides and nucleotides modified with N-methylanthraniloyl (MANT)- or anthraniloyl groups at the 2'(3')-O ribosyl position. MANT-CTP was the most potent EF inhibitor (K<sub>i</sub>, 100 nM) among 16 compounds studied. MANT-nucleotides inhibited EF competitively. Activation of EF by calmodulin resulted in effective fluorescence resonance energy transfer (FRET) from tryptophan and tyrosine residues located in the vicinity of the catalytic site to MANT-ATP, but FRET to MANT-CTP was only small. Mutagenesis studies revealed that Phe586 is crucial for FRET to MANT-ATP and MANT-CTP and that the mutations N583Q, K353A, and K353R differentially alter the inhibitory potencies of MANT-ATP and MANT-CTP. Docking approaches relying on crystal structures of EF indicate similar binding modes of the MANT nucleotides with subtle differences in the region of the nucleobases."

"Like mAC, EF accommodates both purine and pyrimidine nucleotides. The unique preference of EF for the base cytosine offers an excellent starting point for the development of potent and selective EF inhibitors."

The full article can be found at: (H.M. Taha, et. al., "Molecular Analysis of the Interaction of Anthrax Adenylyl Cyclase Toxin, Edema Factor, with 2'(3')-O-(N-(Methyl)anthraniloyl)-Substituted Purine and Pyrimidine Nucleotides". *Molecular Pharmacology*, 2009;75(3):693-703). Link not available.  
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### **SUB-GENOMIC REPLICON AND VIRUS-LIKE PARTICLES OF OMSK HEMORRHAGIC FEVER VIRUS**

Medical Letter on the CDC & FDA  
April 26, 2009

"Omsk hemorrhagic fever virus (OHFV) is a member of the tick-borne encephalitis serocomplex of flaviviruses, and causes hemorrhagic disease in humans. To investigate the molecular mechanisms involved in OHFV pathogenesis, we constructed several subgenomic OHFV replicons containing large

deletions in the structural region.”

“Replicon RNA was introduced into BHK cells by transfection and the production of viral proteins was monitored by IFA. GFP and luciferase genes were inserted into the OHFV replicon, and these reporter genes were expressed in cells harboring replicating replicon RNA. OHFV replicons were packaged into single-round infectious virus-like particles (VLPs) by sequential transfection with replicon RNA and a plasmid expressing the viral structural proteins. Reporter genes were expressed in cells infected with VLPs, and the infection was inhibited by neutralizing antibodies.”

“These replicon and VLP systems will be useful tools for investigating the molecular mechanism of OHFV pathogenicity.”

The full article can be found at: (K. Yoshii, et. al., “Sub-genomic replicon and virus-like particles of Omsk hemorrhagic fever virus”. Archives of Virology, 2009;154(4):573-80). Link not available.

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## **TWO SMALL C-TYPE CYTOCHROMES AFFECT VIRULENCE GENE EXPRESSION IN BACILLUS ANTHRACIS**

Biotech Law Weekly

April 17, 2009

To identify factors that regulate toxin expression, transposon mutagenesis was performed under non-inducing conditions and mutants were isolated that untimely expressed high levels of toxin. A number of these mutations clustered in the haem biosynthetic and cytochrome c maturation pathways. Genetic analysis revealed that two haem-dependent, small c-type cytochromes, CccA and CccB, located on the extracellular surface of the cytoplasmic membrane, regulate toxin gene expression by affecting the expression of the master virulence regulator AtxA. Deregulated AtxA expression in early exponential phase resulted in increased expression of toxin genes in response to loss of the CccA-CccB signalling pathway. This is the first function identified for these two small c-type cytochromes of Bacillus species. Extension of the transposon screen identified a previously uncharacterized protein, BAS3568, highly conserved across many bacterial and archeal species, as involved in cytochrome c activity and virulence regulation.”

“These findings are significant not only to virulence regulation in B. anthracis, but also to analysis of virulence regulation in many pathogenic bacteria and to the study of cytochrome c activity in Gram-positive bacteria.”

The full article can be found at: (A.C. Wilson, et. al., “Two small c-type cytochromes affect virulence gene expression in Bacillus anthracis”. Molecular Microbiology, 2009;72(1):109-23). Link not available.

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