23 July 2009

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 Edition

1. NUCLEOPHILIC REACTIVATION OF SARIN-INHIBITED ACETYLCHOLINESTERASE: A MOLECULAR MODELING STUDY: *"We propose the use of hydrazones and hydrazonates for further evaluation as antidotes for intoxication by chemical warfare agents.."*

2. TRICHOTHECENE MYCOTOXINS ACTIVATE INFLAMMATORY RESPONSE IN HUMAN MACROPHAGES: *"Our results indicate that human macrophages sense trichothecene mycotoxins as a danger signal, which activates caspase-1, and further enables the secretion of IL-1 beta and IL-18 from the LPS-primed cells."*

3. STRUCTURE AND INHIBITION OF THE SARS CORONAVIRUS ENVELOPE PROTEIN ION CHANNEL: *"The channel structure presented herein provides a possible rationale for inhibition, and a platform for future structure-based drug design of this potential pharmacological target."*

4. EXTENSIVE ANTIBODY CROSS-REACTIVITY AMONG INFECTIOUS GRAM-NEGATIVE BACTERIA REVEALED BY PROTEOME MICROARRAY ANALYSIS: *"Thus, our results demonstrate potential biomarkers that are either specific to one strain or common to several species of pathogenic bacteria."*

5. EARLY AND STRONG IMMUNE RESPONSES ARE ASSOCIATED WITH CONTROL OF VIRAL REPLICATION AND RECOVERY IN LASSA VIRUS-INFECTED CYNOMOLGUS MONKEYS: *"Thus, early and strong immune responses and control of viral replication were associated with recovery, whereas fatal infection was characterized by major alterations of the blood formula and, in organs, weak immune responses and uncontrolled viral replication.."*

6. DETECTION OF ORGANOPHOSPHORUS PESTICIDES USING POTENTIOMETRIC ENZYMATIC MEMBRANE BIOSENSOR BASED ON METHYLCELLULOSE IMMOBILIZATION: *"The basic element of this biosensor was a pH electrode modified with an immobilized acetylcholinesterase layer formed by entrapment with methylcellulose, N,N-dimethylformamide, and bovine serum albumin."*

CB Daily Report

Chem-Bio News

NUCLEOPHILIC REACTIVATION OF SARIN-INHIBITED ACETYLCHOLINESTERASE: A MOLECULAR MODELING STUDY
Journal of Technology & Science
July 26, 2009

"Oximes have been used as reactivators for organophosphorus-inhibited acetylcholinesterase (AChE). However, it is still not clear why oximes are more efficient than other nucleophiles, since the reactivation consists of a simple nucleophilic substitution."

"In an attempt to answer this question, we have modeled the sarin-inhibited AChE reactivation by other

nucleophiles (with and without the so-called alpha-effect) by applying the B3LYP/6-311G(d,p) level of theory. We have concluded that oximes reactivate AChE by a three-step mechanism in opposition to the four-step processes of the other modeled nucleophiles. In addition, our model suggests that oximes react with AChE in the deprotonated form (oximate). Our results also indicate that other nucleophiles may be used as AChE reactivators."

"We propose the use of hydrazones and hydrazonates for further evaluation as antidotes for intoxication by chemical warfare agents."

The full article can be found at: (R.T. Delfino, et. al., "Nucleophilic Reactivation of Sarin-Inhibited Acetylcholinesterase: A Molecular Modeling Study". Journal of Physical Chemistry B, 2009;113(24):8402-8411). Link not available.
Return to Top

TRICHOTHECENE MYCOTOXINS ACTIVATE INFLAMMATORY RESPONSE IN HUMAN MACROPHAGES
Immunotherapy Weekly
July 15, 2009

"Trichothecene mycotoxins form one major group of toxins, which possibly contribute to the illnesses. Stachybotrys chartarum is a well-known, but also controversial damp building mold and many strains of this mold are capable of producing trichothecenes. In this report, we have examined the effect of S. chartarum and trichothecene mycotoxins on the proinflammatory cytokine response in human macrophages. As a result, satratoxin-positive S. chartarum activated inflammasome-associated caspase-1, which is needed for proteolytic processing of IL-1 beta and IL-18. Furthermore, purified trichothecene mycotoxins, roridin A, verrucarin A, and T-2 toxin activated caspase-1, and these mycotoxins also strongly enhanced LPS-dependent secretion of IL-1 beta and IL-18. The satratoxin-positive strain of S. chartarum and the trichothecenes also triggered the activation of caspase-3, which is an effector caspase of apoptosis. Satratoxin-negative S. chartarum was not able to activate either caspase-1 or caspase-3."

"Our results indicate that human macrophages sense trichothecene mycotoxins as a danger signal, which activates caspase-1, and further enables the secretion of IL-1 beta and IL-18 from the LPS-primed cells."

The full article can be found at: (P. Kankkunen, et. al., "Trichothecene Mycotoxins Activate Inflammatory Response in Human Macrophages". Journal of Immunology, 2009;182(10):6418-6425). Link not available.
Return to Top

STRUCTURE AND INHIBITION OF THE SARS CORONAVIRUS ENVELOPE PROTEIN ION CHANNEL
By Konstantin Pervushin, Edward Tan, Krupakar Parthasarathy, Xin Lin, Feng Li Jiang, Dejie Yu, Ardcharaporn Vararattanavech, Tuck Wah Soong, Ding Xiang Liu, Jaume Torres
PLos Pathogens
July 10, 2009

"Abstract

The envelope (E) protein from coronaviruses is a small polypeptide that contains at least one α-helical transmembrane domain. Absence, or inactivation, of E protein results in attenuated viruses, due to alterations in either virion morphology or tropism. Apart from its morphogenetic properties, protein E has been reported to have membrane permeabilizing activity. Further, the drug hexamethylene amiloride (HMA), but not amiloride, inhibited in vitro ion channel activity of some synthetic coronavirus E proteins, and also viral replication. We have previously shown for the coronavirus species responsible

for severe acute respiratory syndrome (SARS-CoV) that the transmembrane domain of E protein (ETM) forms pentameric α-helical bundles that are likely responsible for the observed channel activity. Herein, using solution NMR in dodecylphosphatidylcholine micelles and energy minimization, we have obtained a model of this channel which features regular α-helices that form a pentameric left-handed parallel bundle. The drug HMA was found to bind inside the lumen of the channel, at both the C-terminal and the N-terminal openings, and, in contrast to amiloride, induced additional chemical shifts in ETM. Full length SARS-CoV E displayed channel activity when transiently expressed in human embryonic kidney 293 (HEK-293) cells in a whole-cell patch clamp set-up. This activity was significantly reduced by hexamethylene amiloride (HMA), but not by amiloride. The channel structure presented herein provides a possible rationale for inhibition, and a platform for future structure-based drug design of this potential pharmacological target.

Author Summary

Coronaviruses are viral pathogens that cause a variety of lethal diseases in birds and mammals, and common colds in humans. In 2003, however, an animal coronavirus was able to infect humans and produced severe acute respiratory syndrome (SARS), causing a near pandemic. Such events are likely to reoccur in the future, and new antiviral strategies are necessary. A small coronavirus protein called 'envelope' is important for pathogenesis, affecting the formation of the viral envelope and the distribution of the virus in the body. In vitro studies have shown that synthetic coronavirus envelope proteins have channel activity that in some cases has been inhibited by the drug hexamethylene amiloride, but not by amiloride. In the present paper, we have characterized the structure responsible for this channel activity. We have also determined the binding site of the drug hexamethylene amiloride in the channel, and shown that amiloride has only a mild effect on the NMR signals from the protein. The validity of these results is supported using mammalian cells expressing full length SARS-CoV E, where channel activity was inhibited by hexamethylene amiloride, but only mildly by amiloride. The structural model described for this channel provides a valuable insight into coronavirus envelope protein ion channel activity, and could serve as a platform for the development of novel anti-viral drugs."

The full article can be found at: http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000511;jsessionid=9CE0C5CA9BBE2BEB5ABAE90AEE004220
Return to Top

EXTENSIVE ANTIBODY CROSS-REACTIVITY AMONG INFECTIOUS GRAM-NEGATIVE BACTERIA REVEALED BY PROTEOME MICROARRAY ANALYSIS
Medical Letter on the CDC & FDA
July 26, 2009

"We developed a microarray comprising similar to 70% of the 4066 proteins contained within the Yersinia pestis proteome to identify antibody biomarkers distinguishing plague from infections caused by other bacterial pathogens that may initially present similar clinical symptoms. We first examined rabbit antibodies produced against proteomes extracted from Y. pestis, Burkholderia mallei, Burkholderia cepecia, Burkholderia pseudomallei, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella flexneri, and Escherichia coli, all pathogenic Gram-negative bacteria. These antibodies enabled detection of shared cross-reactive proteins, fingerprint proteins common for two or more bacteria, and signature proteins specific to each pathogen. Recognition by rabbit and non-human primate antibodies involved less than 100 of the thousands of proteins present within the Y. pestis proteome. Further antigen binding patterns were revealed that could distinguish plague from anthrax, caused by the Gram-positive bacterium Bacillus anthracis, using sera from acutely infected or convalescent primates. Thus, our results demonstrate potential biomarkers that are either specific to one strain or common to several species of pathogenic bacteria."

The full article can be found at: (S.L. Keasey, et. al., "Extensive Antibody Cross-reactivity among Infectious Gram-negative Bacteria Revealed by Proteome Microarray Analysis". Molecular & Cellular Proteomics, 2009;8(5):924-935). Link not available.
Return to Top

EARLY AND STRONG IMMUNE RESPONSES ARE ASSOCIATED WITH CONTROL OF VIRAL REPLICATION AND RECOVERY IN LASSA VIRUS-INFECTED CYNOMOLGUS MONKEYS
Medical Letter on the CDC & FDA
July 19, 2009

"We followed virological, pathological, and immunological markers associated with fatal and nonfatal Lassa virus infection of cynomolgus monkeys. The clinical picture was characterized by fever, weight loss, depression, and acute respiratory syndrome. Transient thrombocytopenia and lymphopenia, lymphadenopathy, splenomegaly, infiltration of mononuclear cells, and alterations of the liver, lungs, and endothelia were observed. Survivors exhibited fewer lesions and a lower viral load than nonsurvivors. Although all animals developed strong humoral responses, antibodies appeared more rapidly in survivors and were directed against GP(1), GP(2), and NP. Type I interferons were detected early after infection in survivors but only during the terminal stages in fatalities. The mRNAs for CXCL10 (IP-10) and CXCL11 (I-TAC) were abundant in peripheral blood mononuclear cells and lymph nodes from infected animals, but plasma interleukin-6 was detected only in fatalities. In survivors, high activated-monocyte counts were followed by a rise in the total number of circulating monocytes. Activated T lymphocytes circulated in survivors, whereas T-cell activation was low and delayed in fatalities. In vitro stimulation with inactivated Lassa virus induced activation of T lymphocytes from all infected monkeys, but only lymphocytes from survivors proliferated."

"Thus, early and strong immune responses and control of viral replication were associated with recovery, whereas fatal infection was characterized by major alterations of the blood formula and, in organs, weak immune responses and uncontrolled viral replication.."

The full article can be found at: (S. Baize, et. al., "Early and Strong Immune Responses Are Associated with Control of Viral Replication and Recovery in Lassa Virus-Infected Cynomolgus Monkeys". Journal of Virology, 2009;83(11):5890-5903). Link not available.
Return to Top

DETECTION OF ORGANOPHOSPHORUS PESTICIDES USING POTENTIOMETRIC ENZYMATIC MEMBRANE BIOSENSOR BASED ON METHYLCELLULOSE IMMOBILIZATION
Electronics Newsweekly
July 22, 2009

"In this research, a new potentiometric enzymatic membrane biosensor for the detection of organophosphorus pesticides (OPs) was constructed. The basic element of this biosensor was a pH electrode modified with an immobilized acetylcholinesterase layer formed by entrapment with methylcellulose, N,N-dimethylformamide, and bovine serum albumin."

"The response characteristics of the biosensor were measured and discussed. It was shown that there is a good linear relationship between the inhibition rate and the negative logarithm of OPs concentration in the range from 10(-7) to 10(-5) mol/L, with the detection limits of 10(-7) mol/L for the five pesticides. Moreover, the biosensor could resist the disturbances of below 10(-6) mol/L of Cu2+ and Pb2+, and below 10(-5) mol/L of Cd2+. In addition, the measurement results obtained by the biosensor method were in good agreement with those obtained by the gas chromatography method."

The full article can be found at: (J.Z. Zhang, et. al., "Detection of Organophosphorus Pesticides Using Potentiometric Enzymatic Membrane Biosensor Based on Methylcellulose Immobilization". Analytical Sciences, 2009;25(4):511-515). Link not available.
Return to Top

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