

16 April 2009

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Chem-Bio News – S&T Edition

1. ANTHRAX LETHAL TOXIN IMPAIRS IL-8 EXPRESSION IN EPITHELIAL CELLS THROUGH INHIBITION OF HISTONE H3 MODIFICATION: : *"Our results suggest that B. anthracis represses the immune response, in part by altering chromatin accessibility of IL-8 promoter to NF- κ B in epithelial cells. This epigenetic reprogramming, in addition to previously reported effects of LT, may represent an efficient strategy used by B. anthracis for invading the host."*

2. CASPASE-7 ACTIVATION BY THE NLRC4/IPAF INFLAMMASOME RESTRICTS LEGIONELLA PNEUMOPHILA INFECTION: *"These results reveal a new mechanism for caspase-7 activation downstream of the Nlrc4 inflammasome and present a novel biological role for caspase-7 in host defense against an intracellular bacterium."*

3. FUNCTION-ORIENTED SYNTHESIS APPLIED TO THE ANTI-BOTULINUM NATURAL PRODUCT TOSENDANIN: : *"From these studies a new synthetic strategy is put forth allowing access to a 4-acetoxy CD fragment analogue (14) of toosendanin, which was achieved from mesityl oxide and acetylacetone in 14 steps."*

4. THE INFLUENCE OF LIGAND VALENCY ON AGGREGATION MECHANISMS FOR INHIBITING BACTERIAL TOXINS: : *"Structural studies employing atomic force microscopy have revealed that a divalent inhibitor induces head-to-head dimerization of the protein toxin en route to higher aggregates."*

5. SARDASHT-IRAN COHORT STUDY OF CHEMICAL WARFARE VICTIMS : DESIGN AND METHODS: *"Sardasht-Iran Cohort Study is a comprehensive historical cohort study on Sardasht chemical victims' population which was designed to find out the long-term complications of sulfur mustard exposure and the basic mechanisms underlying clinical manifestations."*

6. MICROFLUIDIC DEVICE USING CHEMILUMINESCENCE AND A DNA-ARRAYED THIN FILM TRANSISTOR PHOTOSENSOR FOR SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPING OF PCR AMPLICONS FROM WHOLE BLOOD: *"This work describes a novel microfluidic device using a thin film transistor (TFT) photosensor integrating a microfluidic channel, a DNA chip platform, and a photodetector for the discrimination of single nucleotide polymorphisms (SNPs). A DNA-arrayed TFT photosensor was used as a DNA chip platform and photo detecting device."*

7. NEW INSIGHTS INTO HOW SARS PATHOGEN INFECTS HOST: *"Now, Cornell researchers have discovered key properties in coronaviruses that help explain how these viruses invade their hosts and cross species barriers."*

8. CENTRAL ROLES FOR IL-2 AND MCP-1 FOLLOWING INTRANASAL EXPOSURE TO SEB: A NEW MOUSE MODEL: *"Central roles for IL-2 and MCP-1 following intranasal exposure to SEB: A new mouse model."*

9. EXPERIMENTAL STUDY OF DISPERSION AND DEPOSITION OF EXPIRATORY AEROSOLS IN AIRCRAFT CABINS AND IMPACT ON INFECTIOUS DISEASE TRANSMISSION: *"Results show that the cough jet could bring significant amount of aerosols forward to the row of seats ahead of the cougher and the aerosols were then dispersed by the bulk air movements in the lateral direction."*

10. INVESTIGATION TOWARDS BIVALENT CHEMICALLY DEFINED GLYCOCONJUGATE IMMUNOGENS PREPARED FROM ACID-DETOXIFIED LIPOPOLYSACCHARIDE OF VIBRIO CHOLERAЕ O1, SEROTYPE INABA: *"Taken together, these results encourage further investigation towards the development of potent pmLPS-based neoglycoconjugate immunogens, fully aware of the*

challenge faced in the development of a cholera vaccine that will provide efficient serogroup coverage."

11. TWO SMALL C-TYPE CYTOCHROMES AFFECT VIRULENCE GENE EXPRESSION IN BACILLUS ANTHRACIS: "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."

CB Daily Report

Chem-Bio News

ANTHRAX LETHAL TOXIN IMPAIRS IL-8 EXPRESSION IN EPITHELIAL CELLS THROUGH INHIBITION OF HISTONE H3 MODIFICATION

By Benoit Raymond, Eric Batsche3, Florence Boutillon, Yong-Zheng Wu, Dominique Leduc, Viviane Balloy, Eloïse Raoust, Christian Muchardt, Pierre L. Goossens, Lhousseine Touqui
PloS Pathogens
April 3, 2009

"Lethal toxin (LT) is a critical virulence factor of *Bacillus anthracis*, the etiological agent of anthrax, whose pulmonary form is fatal in the absence of treatment. Inflammatory response is a key process of host defense against invading pathogens. We report here that intranasal instillation of a *B. anthracis* strain bearing inactive LT stimulates cytokine production and polymorphonuclear (PMN) neutrophils recruitment in lungs. These responses are repressed by a prior instillation of an LT preparation. In contrast, instillation of a *B. anthracis* strain expressing active LT represses lung inflammation. The inhibitory effects of LT on cytokine production are also observed in vitro using mouse and human pulmonary epithelial cells. These effects are associated with an alteration of ERK and p38-MAPK phosphorylation, but not JNK phosphorylation. We demonstrate that although NF- κ B is essential for IL-8 expression, LT downregulates this expression without interfering with NF- κ B activation in epithelial cells. Histone modifications are known to induce chromatin remodelling, thereby enhancing NF- κ B binding on promoters of a subset of genes involved in immune response. We show that LT selectively prevents histone H3 phosphorylation at Ser 10 and recruitment of the p65 subunit of NF- κ B at the IL-8 and KC promoters. Our results suggest that *B. anthracis* represses the immune response, in part by altering chromatin accessibility of IL-8 promoter to NF- κ B in epithelial cells. This epigenetic reprogramming, in addition to previously reported effects of LT, may represent an efficient strategy used by *B. anthracis* for invading the host."

The full article can be found at: <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000359;jsessionid=0BF90A3081FD0A047F890CF1208AFBCC>

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CASPASE-7 ACTIVATION BY THE NLRC4/IPAF INFLAMMASOME RESTRICTS LEGIONELLA PNEUMOPHILA INFECTION

By Anwari Akhter1, Mikhail A. Gavrillin1, Laura Frantz1, Songcerae Washington1, Cameron Ditty1, Dominique Limoli1, Colby Day1, Anasuya Sarkar1, Christie Newland1, Jonathan Butchar1, Clay B. Marsh1, Mark D. Wewers1, Susheela Tridandapani1, Thirumala-Devi Kanneganti2*, Amal O. Amer
PloS Pathogens
April 3, 2009

"*Legionella pneumophila* (*L. pneumophila*), the causative agent of a severe form of pneumonia called Legionnaires' disease, replicates in human monocytes and macrophages. Most inbred mouse strains are restrictive to *L. pneumophila* infection except for the A/J, *Nlrc4*^{-/-} (*Ipaf*^{-/-}), and caspase-1^{-/-} derived macrophages. Particularly, caspase-1 activation is detected during *L. pneumophila* infection of murine macrophages while absent in human cells. Recent in vitro experiments demonstrate that

caspase-7 is cleaved by caspase-1. However, the biological role for caspase-7 activation downstream of caspase-1 is not known. Furthermore, whether this reaction is pertinent to the apoptosis or to the inflammation pathway or whether it mediates a yet unidentified effect is unclear. Using the intracellular pathogen *L. pneumophila*, we show that, upon infection of murine macrophages, caspase-7 was activated downstream of the Nlrc4 inflammasome and required caspase-1 activation. Such activation of caspase-7 was mediated by flagellin and required a functional Naip5. Remarkably, mice lacking caspase-7 and its macrophages allowed substantial *L. pneumophila* replication. Permissiveness of caspase-7^{-/-} macrophages to the intracellular pathogen was due to defective delivery of the organism to the lysosome and to delayed cell death during early stages of infection. These results reveal a new mechanism for caspase-7 activation downstream of the Nlrc4 inflammasome and present a novel biological role for caspase-7 in host defense against an intracellular bacterium."

The full article can be found at: <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000361;jsessionid=0BF90A3081FD0A047F890CF1208AFBCC>

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FUNCTION-ORIENTED SYNTHESIS APPLIED TO THE ANTI-BOTULINUM NATURAL PRODUCT TOSENDANIN

Biotech Week
April 8, 2009

"The natural product tosendanin is a traditional Chinese medicine which has been reported to have anti-botulinum properties in animal models."

"To establish what chemical functionalities are necessary for the anti-botulinum properties found within tosendanin, a study was initiated with the goal of using function-oriented synthesis (FOS) as a strategy to begin to unravel tosendanin's powerful anti-botulinum properties. From these studies a new synthetic strategy is put forth allowing access to a 4-acetoxy CD fragment analogue (14) of tosendanin, which was achieved from mesityl oxide and acetylacetone in 14 steps."

The full article can be found at: (Y. Nakai, et. al., "Function-oriented synthesis applied to the anti-botulinum natural product tosendanin". *Bioorganic & Medicinal Chemistry*, 2009;17(3):1152-1157). Link not available.

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THE INFLUENCE OF LIGAND VALENCY ON AGGREGATION MECHANISMS FOR INHIBITING BACTERIAL TOXINS

Malaria Weekly
April 13, 2009

"Divalent and tetravalent analogues of ganglioside GM1 are potent inhibitors of cholera toxin and *Escherichia coli* heat-labile toxin, However, they show little increase in inherent affinity when compared to the corresponding monovalent carbohydrate ligand Analytical ultracentrifugation and dynamic light scattering have been used to demonstrate that the multivalent inhibitors induce protein aggregation and the formation of space-filling networks."

"This aggregation process appears to arise when using ligands that do not match the valency of the protein receptor. While it is generally accepted that multivalency is an effective strategy for increasing the activity of inhibitors, here we show that the valency of the inhibitor also has a dramatic effect on the kinetics of aggregation and the stability of intermediate protein complexes."

"Structural studies employing atomic force microscopy have revealed that a divalent inhibitor induces head-to-head dimerization of the protein toxin en route to higher aggregates."

The full article can be found at: (C. Sisu, et. al., "The Influence of Ligand Valency on Aggregation

Mechanisms for Inhibiting Bacterial Toxins". *Chembiochem*, 2009;10(2):329-337). Link not available.
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SARDASHT-IRAN COHORT STUDY OF CHEMICAL WARFARE VICTIMS : DESIGN AND METHODS

By Ghazanfari Tooba ; Faghihzadeh Soghrat; Aragizadeh Hassan; Soroush Mohammad-Reza; Yaraee Roya; Zuhair Mohammad Hassan; Foroutan Abbas; Vaez-Mahdavi Mohammad-Reza; Javadi Mohammad-Ali Moaiedmohseni Sakine; Azizi Fereidoun; Panahi Yunes; Mostafaie Ali; Ghasemi Hassan; Shams Jaleleddin; Pourfarzam Shahrya; Jalali-Nadoushan Mohammad-Reza (17) ; Fallahi Faramarz ; Ebtekar Massoumeh; Davoudi Seyyed-Masoud ; Ghazanfari Zeinab ; Ardestani Sussan K. (20) ; Shariat-Panahi Shamsa; Moin Athar; Rezaei Abbas; Kariminia Amina; Ajdary Soheila; Mahmoudi Mahmoud; Roshan Rasoul; Ghaderi Sulayman ; Babai Mahmoud; Naghizadeh Mohammad-Mehdi ; Ghanei Mostafa
Archives of Iranian Medicine
2009

“Abstract

Background: Insights into long-term clinical consequences of sulfur mustard have emerged from some investigations but less is known about the basic and molecular mechanisms of these complications. Sardasht-Iran Cohort Study is a comprehensive historical cohort study on Sardasht chemical victims' population which was designed to find out the long-term complications of sulfur mustard exposure and the basic mechanisms underlying clinical manifestations. This paper describes the design and methodology of Sardasht-Iran Cohort Study. Methods: In Sardasht-Iran Cohort Study, 500 individuals including 372 subjects from Sardasht, as the exposed group, and 128 subjects from Rabat, as the unexposed age-matched control group were evaluated. The exposed group was divided into two groups based on the severity of clinical complications at the time of exposure. Different samples including blood, sputum, saliva, tear, urine, and semen were collected for immunologic, hematologic, biochemical, and other laboratory analysis. Data were gathered from medical records, clinical examinations, laboratory tests, and questionnaires for psychological and lifestyle situations. Conclusion: The important distinctions setting this study apart from the previous ones are discussed. The Sardasht-Iran Cohort Study provides important information on various aspects of long- term consequences of sulfur mustard exposure. This database will provide a better position to suggest guidelines for the diagnosis, treatment, and prevention of delayed complications In the patients exposed to sulfur mustard.”

The full article can be found at: <http://cat.inist.fr/?aModele=afficheN&cpsidt=21097845>
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MICROFLUIDIC DEVICE USING CHEMILUMINESCENCE AND A DNA-ARRAYED THIN FILM TRANSISTOR PHOTODIODE FOR SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPING OF PCR AMPLICONS FROM WHOLE BLOOD

By Keiichi Hatakeyama, Tsuyoshi Tanaka, Masahiro Sawaguchi, Akihito Iwadate, Yasushi Mizutani, Kazuhiro Sasaki, Naofumi Tateishi and Tadashi Matsunaga
Lab on a Chip (Royal Society of Chemistry, UK)
2009

“This work describes a novel microfluidic device using a thin film transistor (TFT) photodiode integrating a microfluidic channel, a DNA chip platform, and a photodiode for the discrimination of single nucleotide polymorphisms (SNPs). A DNA-arrayed TFT photodiode was used as a DNA chip platform and photo detecting device. Chemiluminescence was used for DNA sensing because chemiluminescence provides higher sensitivity and requires simpler instrumentation than fluorescence methods. The SNP of biotinylated target DNA was detected based on chemiluminescence by using horse radish peroxidase-conjugated streptavidin. The lower detection limit for a model biotinylated oligonucleotide (63-mer) was 0.5 nM, much lower than expected DNA concentrations in a practical application of this device. Furthermore, SNP detection in the aldehyde dehydrogenase 2 gene was successfully achieved using DNA-arrayed TFT photodiode without DNA extraction and DNA purification

using PCR products. The assay was completed in less than one hour. Our technology will be a promising approach to developing a miniaturized, disposable DNA chip with high sensitivity."

The full article can be found at: <http://www.rsc.org/Publishing/Journals/LC/article.asp?doi=b817427j>
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NEW INSIGHTS INTO HOW SARS PATHOGEN INFECTS HOST

By Krishna Ramanujan
Physorg.com
April 14, 2009

"When Severe Acute Respiratory Syndrome (SARS) first appeared in 2003, international cooperation helped contain the virulent coronavirus, which caused respiratory illness in more than 8,000 people and killed almost 10 percent of them. Better understanding of such viruses will help control similar diseases when they strike again.

Now, Cornell researchers have discovered key properties in coronaviruses that help explain how these viruses invade their hosts and cross species barriers. The SARS virus, for example, originated in bats, jumped to civets (weasel-like mammals) in Chinese markets and then to humans. Other coronaviruses cause the common cold and croup in humans.

The researchers have discovered two sites -- called cleavage sites -- where a key structural protein on the virus gets split, activating a process that allows the virus to enter a host cell. They report their findings online in the Proceedings of the National Academy of Sciences. One cleavage site was known to exist, but studies of a mutated vaccine strain of another highly virulent avian coronavirus, known as infectious bronchitis virus (IBV), revealed a second cleavage site. This discovery led Cornell researchers to search for a second cleavage site in the SARS virus, which they found in exactly the same location as in IBV."

The full article can be found at: <http://www.physorg.com/news158947206.html>
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CENTRAL ROLES FOR IL-2 AND MCP-1 FOLLOWING INTRANASAL EXPOSURE TO SEB: A NEW MOUSE MODEL

Food & Farm Week
April 16, 2009

"Murine models for bacterial superantigens like staphylococcal enterotoxin B (SEB) have to date been rather cumbersome. The reasons include: (1) necessary use of potentiating agents such as actinomycin D, D-galactosamine, lipopolysaccharide (LPS), or viruses; (2) high toxin amounts required to elicit effects; and/or (3) generation of phenotypic-stable transgenic animals."

"Our study employed readily available C3H/HeJ (TLR4 negative, LPS-nonresponsive) mice with intranasal and intraperitoneal administration of low microgram quantities of SEB. These animals responded to SEB with severe lung inflammation and hypothermia, culminating in death. A survey of cytokines/chemokines in sera and lungs after lethal intoxication revealed that monocyte chemoattractant protein-1 and interleukin-2 were associated with effects in this model. In contrast, SEB had minimal effects upon congenic (TLR4 positive, LPS-responsive) C3H/OuJ mice."

"Lethality of SEB in C3H/HeJ mice was neutralized with SEB-specific antibodies, suggesting potential utility of this model for future therapeutic studies."

The full article can be found at: (L.M. Huzella, et. al., "Central roles for IL-2 and MCP-1 following intranasal exposure to SEB: A new mouse model". Research in Veterinary Science, 2009;86(2):241-247). Link not available.

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EXPERIMENTAL STUDY OF DISPERSION AND DEPOSITION OF EXPIRATORY AEROSOLS IN AIRCRAFT CABINS AND IMPACT ON INFECTIOUS DISEASE TRANSMISSION

Science Letter

April 14, 2009

"The dispersion and deposition characteristics of polydispersed expiratory aerosols were investigated in an aircraft cabin mockup to study the transmission of infectious diseases. The airflow was characterized by particle image velocimetry (PIV) measurements."

"Aerosol dispersion was measured by the Interferometric Mie Imaging (IMI) method combined with an aerosol spectrometer. Deposition was investigated using the fluorescent dye technique. Downward air currents were observed near the seats next to the side walls while upward airflows were observed near other seats. The downward airflow showed some effects on suppressing the dispersion of aerosols expelled by the passenger sitting in the window seat. Results show that the cough jet could bring significant amount of aerosols forward to the row of seats ahead of the cougher and the aerosols were then dispersed by the bulk air movements in the lateral direction. The aerosols expelled from a cough took 20-30 s to reach the breathing zones of the passengers seated within two rows from the cougher. Increasing the ventilation rate improved the dilution and reduced the aerosol exposure to passengers seated close to the source, but the aerosol dispersion increased, which heightened the exposure to passengers seated further away. 60-70% of expiratory aerosols in mass were deposited, with significant portions on surfaces close to the source, suggesting that disease transmission risk via indirect contact in addition to airborne risk is possible."

"The physical transport processes of expiratory aerosols could be used to shed insights on some epidemiological observations on in-flight transmission of certain infectious diseases."

The full article can be found at: (G.N.S. To, et. al., "Experimental Study of Dispersion and Deposition of Expiratory Aerosols in Aircraft Cabins and Impact on Infectious Disease Transmission". Aerosol Science and Technology, 2009;43(5):466-485). Link not available.

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INVESTIGATION TOWARDS BIVALENT CHEMICALLY DEFINED GLYCOCONJUGATE IMMUNOGENS PREPARED FROM ACID-DETOXIFIED LIPOPOLYSACCHARIDE OF VIBRIO CHOLERAE O1, SEROTYPE INABA

Biotech Law Weekly

April 10, 2009

"A free amino group present on the acid-detoxified lipopolysaccharide (pmLPS) of *V. cholerae* O1 serotype Inaba was investigated for site-specific conjugation. Chemoselective pmLPS biotinylation afforded the corresponding mono-functionalized derivative, which retained antigenicity."

"Thus, pmLPS was bound to carrier proteins using thioether conjugation chemistry. Induction of an anti-LPS antibody (Ab) response in BALB/c mice was observed for all conjugates. Interestingly, the sera had vibriocidal activity against both Ogawa and Inaba strains opening the way to a possible bivalent vaccine. However, the level of this Ab response was strongly affected by both the nature of the linker and of the carrier. Furthermore, no switch from IgM to IgG, i.e. from a T cell-independent to a T cell-dependent immune response was detected, a result tentatively explained by the possible presence of free polysaccharide in the formulation."

"Taken together, these results encourage further investigation towards the development of potent pmLPS-based neoglycoconjugate immunogens, fully aware of the challenge faced in the development of a cholera vaccine that will provide efficient serogroup coverage."

The full article can be found at: (C. Grandjean, et. al., "Investigation towards bivalent chemically defined glycoconjugate immunogens prepared from acid-detoxified lipopolysaccharide of *Vibrio cholerae* O1, serotype Inaba". *Glycoconjugate Journal*, 2009;26(1):41-55). Link not available.

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

Biotech Week

April 15, 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).

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