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## **Chem-Bio News – S&T Edition**

- 1. CD14-MAC-1 INTERACTIONS IN BACILLUS ANTHRACIS SPORE INTERNALIZATION BY MACROPHAGES:** *"Additionally, after B. anthracis spore challenge of CD14(-/-) mice, interference with the CD14-mediated signaling pathways results in increased mortality."*
- 2. COWPOX VIRUS EXPRESSES A NOVEL ANKYRIN REPEAT NF-KAPPA B INHIBITOR THAT CONTROLS INFLAMMATORY CELL INFLUX INTO VIRUS-INFECTED TISSUES AND IS CRITICAL FOR VIRUS PATHOGENESIS:** *"These results indicate that members of this ANK repeat family are utilized specifically by pathogenic orthopoxviruses to repress the NF-kappa B signaling pathway at tissue sites of virus replication in situ."*
- 3. HIGH-AFFINITY LAMPREY VLRA AND VLRA MONOCLONAL ANTIBODIES:** *"VLRs [variable lymphocyte receptors] may be useful natural single-chain alternatives to conventional antibodies for biotechnology applications."*
- 4. GENOTYPING OF INDIAN YERSINIA PESTIS STRAINS BY MLVA AND REPETITIVE DNA SEQUENCE BASED PCRS:** *"Thus ERIC-PCR appears to have the potential to be used as a molecular marker in the molecular epidemiological investigations of plague."*
- 5. CELL ENVELOPE PERTURBATION INDUCES OXIDATIVE STRESS AND CHANGES IN IRON HOMEOSTASIS IN VIBRIO CHOLERA:** *"We propose that many types of extracytoplasmic stresses, caused either by genetic alterations of outer membrane constituents or by chemical or physical damage to the cell envelope, induce common signaling pathways that ultimately lead to internal oxidative stress and misregulation of iron homeostasis."*
- 6. GROWTH OF CALCIUM-BLIND MUTANTS OF YERSINIA PESTIS AT 37 DEGREES C IN PERMISSIVE CA2+-DEFICIENT ENVIRONMENTS:** *"The Ca2+-blind yopD phenotype was fully suppressed in a Ca2+-independent background lacking the injectisome-associated inner-membrane component YscV but not peripheral YscK, suggesting that the core translocon energizes YopD."*
- 7. FINDINGS MAY LEAD TO NEW VACCINE STRATEGIES FOR PULMONARY TULAREMIA:** *"Immunologists at the University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh of UPMC and the have found a unique quirk in the way the immune system fends off bacteria called Francisella tularensis, which could lead to vaccines that are better able to prevent tularemia infection of the lungs. Their findings were published today in the early, online version of Immunity."*
- 8. A MODEL OF CARDIOVASCULAR DISEASE GIVING A PLAUSIBLE MECHANISM FOR THE EFFECT OF FRACTIONATED LOW-DOSE IONIZING RADIATION EXPOSURE:** *"There is emerging evidence of excess risk of cardiovascular disease at low radiation doses in various occupationally exposed groups receiving small daily radiation doses. Assuming that they are causal, the mechanisms for effects of chronic fractionated radiation exposures on cardiovascular disease are unclear."*
- 9. NEW EVIDENCE FOR TOXIC EFFECTS OF INHALED NANOTUBES:** *"Further evidence for the asbestos-like effects of carbon nanotubes has emerged from a new study in mice. The study shows for the first time that the tubes reach the outer lining of the lung when inhaled - as asbestos does. But researchers say the results should be interpreted with caution."*

### **CD14-MAC-1 INTERACTIONS IN BACILLUS ANTHRACIS SPORE INTERNALIZATION BY MACROPHAGES**

Food & Drug Law Weekly  
October 23, 2009

"Recognition of BclA by the integrin Mac-1 promotes spore uptake by professional phagocytes, resulting in the carriage of spores to sites of spore germination and bacterial growth in distant lymphoid organs. We show that CD14 binds to rhamnose residues of BclA and acts as a coreceptor for spore binding by Mac-1. In this process, CD14 induces signals involving TLR2 and PI3k that promote inside-out activation of Mac-1, thereby enhancing spore internalization by macrophages. As observed with mice lacking Mac-1, CD14(-/-) mice are also more resistant than wild-type mice to infection by B. anthracis spores. Additionally, after B. anthracis spore challenge of CD14(-/-) mice, interference with the CD14-mediated signaling pathways results in increased mortality."

The full article can be found at: (C. Oliva, et. al., "CD14-Mac-1 interactions in Bacillus anthracis spore internalization by macrophages". Proceedings of the National Academy of Sciences of the United States of America, 2009;106(33):13957-13962). Link not available.

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### **COWPOX VIRUS EXPRESSES A NOVEL ANKYRIN REPEAT NF-KAPPA B INHIBITOR THAT CONTROLS INFLAMMATORY CELL INFLUX INTO VIRUS-INFECTED TISSUES AND IS CRITICAL FOR VIRUS PATHOGENESIS**

Vaccine Weekly  
October 21, 2009

"Many pathogenic orthopoxviruses like variola virus, monkeypox virus, and cowpox virus (CPXV), but not vaccinia virus, encode a unique family of ankyrin (ANK) repeat-containing proteins that interact directly with NF-kappa B1/p105 and inhibit the NF-kappa B signaling pathway. Here, we present the in vitro and in vivo characterization of the targeted gene knockout of this novel NF-kappa B inhibitor in CPXV."

"Our results demonstrate that the vCpx-006KO uniquely induces a variety of NF-kappa B-controlled proinflammatory cytokines from infected myeloid cells, accompanied by a rapid phosphorylation of the I kappa B kinase complex and subsequent degradation of the NF-kappa B cellular inhibitors I kappa B alpha and NF-kappa B1/p105. Moreover, the vCpx-006KO virus was attenuated for virulence in mice and induced a significantly elevated cellular inflammatory process at tissue sites of virus replication in the lung."

"These results indicate that members of this ANK repeat family are utilized specifically by pathogenic orthopoxviruses to repress the NF-kappa B signaling pathway at tissue sites of virus replication in situ."

The full article can be found at: (M.R. Mohamed, et. al., "Cowpox Virus Expresses a Novel Ankyrin Repeat NF-kappa B Inhibitor That Controls Inflammatory Cell Influx into Virus-Infected Tissues and Is Critical for Virus Pathogenesis". Journal of Virology, 2009;83(18):9223-9236). Link not available.

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### **HIGH-AFFINITY LAMPREY VLRA AND VLRA MONOCLONAL ANTIBODIES**

Drug Week  
October 16, 2009

"Although VLRs represent the only known adaptive immune system not based on Ig, little is known about their antigen-binding properties. Here we report robust plasma VLRB responses of lamprey immunized with hen egg lysozyme and beta-galactosidase (beta-gal), demonstrating adaptive immune responses against soluble antigens. To isolate monoclonal VLRs, we constructed large VLR libraries from antigen-stimulated and naive animals in a novel yeast surface-display vector, with the VLR C-terminally fused to the yeast Flo1p surface anchor. We cloned VLRB binders of lysozyme, beta-gal, cholera toxin subunit B, R-phycoerythrin, and B-trisaccharide antigen, with dissociation constants up to the single-digit picomolar range, equivalent to those of high-affinity IgG antibodies. We also isolated from a single lamprey 13 anti-lysozyme VLRA clones with affinities ranging from low nanomolar to mid-picomolar. All of these VLRA clones were closely related in sequence, differing at only 15 variable codon positions along the 244-residue VLR diversity region, which augmented antigen-binding affinity up to 100-fold. Thus, VLRs can provide a protective humoral antipathogen shield. Furthermore, the broad range of nominal antigens that VLRs can specifically bind, and the affinities achieved, indicate a functional parallelism between LRR-based and Ig-based antibodies."

"VLRs may be useful natural single-chain alternatives to conventional antibodies for biotechnology applications."

The full article can be found at: (S. Tasumi, et. al., "High-affinity lamprey VLRA and VLRB monoclonal antibodies". Proceedings of the National Academy of Sciences of the United States of America, 2009;106(31):12891-12896). Link not available.

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## **GENOTYPING OF INDIAN YERSINIA PESTIS STRAINS BY MLVA AND REPETITIVE DNA SEQUENCE BASED PCRS**

Proteomics Weekly  
October 19, 2009

"India experienced two plague outbreaks in Gujarat and Maharastra during 1994 and then in the Shimla district of Himachal Pradesh during 2002. *Yersinia pestis* strains recovered from rodents and pneumonic patients during the 1994 outbreaks, pneumonic patients from the 2002 Shimla outbreak and rodents trapped on the Deccan Plateau during a surveillance activity carried out in 1998 were characterized by MLVA, ERIC-PCR and ERIC-BOX-PCR."

"MLVA genotyping of Indian *Y. pestis* strains revealed strains of 2 *Orientalis*, 1 *Mediaevalis* and 1 *Antiqua* genotypes distributed in three distinct branches corresponding to their biovar. The *Orientalis* genotype strains recovered from the 1994 outbreaks and 1998 surveillance activity clustered in one branch while the *Antiqua* biovar strains from the Shimla outbreak and the *Mediaevalis* strain recovered from a rodent trapped on the Deccan Plateau region during surveillance formed the other branches. The *Orientalis Y. pestis* strains recovered from rodents and patients from the 1994 plague outbreaks exhibited similar MLVA, ERIC-PCR and ERIC-BOX-PCR profiles and these were closely related to the *Orientalis* strains recovered from the rodents trapped on the Deccan Plateau. These data provide evidence for the possible linkage between the *Y. pestis* strains resident in the endemic region and those that were associated with the 1994 plague outbreaks. *Mediaevalis* and *Antiqua* biovars also were recovered from the environmental reservoir on the Deccan Plateau and from the pneumonic patients of 2002 plague outbreak. Therefore, as in Central Asian and African regions, *Antiqua* and *Mediaevalis* biovars seem to be well established in the Indian subcontinent as well. ERIC-PCR DNA fingerprinting delineated genotypes similar to those defined by MLVA."

"Thus ERIC-PCR appears to have the potential to be used as a molecular marker in the molecular epidemiological investigations of plague."

The full article can be found at: (J.J. Kingston, et. al., "Genotyping of Indian *Yersinia pestis* strains by MLVA and repetitive DNA sequence based PCRS". *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 2009;96(3):303-312). Link not available.

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## **CELL ENVELOPE PERTURBATION INDUCES OXIDATIVE STRESS AND CHANGES IN IRON HOMEOSTASIS IN VIBRIO CHOLERA**

Biotech Law Weekly  
October 16, 2009

"The *Vibrio cholerae* type II secretion (T2S) machinery is a multiprotein complex that spans the cell envelope. When the T2S system is inactivated, cholera toxin and other exoproteins accumulate in the periplasmic compartment."

"Additionally, loss of secretion via the T2S system leads to a reduced growth rate, compromised outer membrane integrity, and induction of the extracytoplasmic stress factor RpoE (A.E. Sikora, S. R. Lybarger, and M. Sandkvist, *J. Bacteriol.* 189:8484-8495, 2007). In this study, gene expression profiling reveals that inactivation of the T2S system alters the expression of genes encoding cell envelope components and proteins involved in central metabolism, chemotaxis, motility, oxidative stress, and iron storage and acquisition. Consistent with the gene expression data, molecular and biochemical analyses indicate that the T2S mutants suffer from internal oxidative stress and increased levels of intracellular ferrous iron. By using a *tolA* mutant of *V. cholerae* that shares a similar compromised membrane phenotype but maintains a functional T2S machinery, we show that the formation of radical oxygen species, induction of oxidative stress, and changes in iron physiology are likely general responses to cell envelope damage and are not unique to T2S mutants. Finally, we demonstrate that disruption of the *V. cholerae* cell envelope by chemical treatment with polymyxin B similarly results in induction of the RpoE-mediated stress response, increased sensitivity to oxidants, and a change in iron metabolism."

"We propose that many types of extracytoplasmic stresses, caused either by genetic alterations of outer membrane constituents or by chemical or physical damage to the cell envelope, induce common signaling pathways that ultimately lead to internal oxidative stress and misregulation of iron homeostasis."

The full article can be found at: (A.E. Sikora, et. al., "Cell Envelope Perturbation Induces Oxidative Stress and Changes in Iron Homeostasis in *Vibrio cholera*". *Journal of Bacteriology*, 2009;191(17):5398-5408). Link not available.

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## **GROWTH OF CALCIUM-BLIND MUTANTS OF YERSINIA PESTIS AT 37 DEGREES C IN PERMISSIVE CA<sup>2+</sup>-DEFICIENT ENVIRONMENTS**

Biotech Week  
October 21, 2009

"Cells of wild-type *Yersinia pestis* exhibit a low-calcium response (LCR) defined as bacteriostasis with expression of a pCD-encoded type III secretion system (T3SS) during cultivation at 37 degrees C without added Ca<sup>2+</sup> versus vegetative growth with downregulation of the T3SS with Ca<sup>2+</sup> ( $\geq 2.5$  mM). Bacteriostasis is known to reflect cumulative toxicity of Na<sup>+</sup>, L-glutamic acid and culture pH; control of these variables enables full-scale growth ('rescue') in the absence of Ca<sup>2+</sup>."

"Several T3SS regulatory proteins modulate the LCR, because their absence promotes a Ca<sup>2+</sup>-blind phenotype in which growth at 37 C ceases and the T3SS is constitutive even with added Ca<sup>2+</sup>. This study analysed the connection between the LCR and Ca<sup>2+</sup> by determining the response of selected Ca<sup>2+</sup>-blind mutants grown in Ca<sup>2+</sup>-deficient rescue media containing Na<sup>+</sup> Plus L-glutamate (pH 5.5), where the T3SS is not expressed, L-glutamate alone (pH 6.5), where L-aspartate is fully catabolized, and Na<sup>+</sup> alone (pH 9.0), where the electrogenic sodium pump NADH : ubiquinone oxidoreductase becomes activated. All three conditions supported essentially full-scale Ca<sup>2+</sup>-independent growth at 37 degrees C of wild-type *Y. pestis* as well as *IcrG* and *yopN* mutants (possessing a complete but dysregulated T3SS), indicating that bacteriostasis reflects a Na<sup>+</sup>-dependent lesion in bioenergetics. In contrast, mutants lacking the negative regulator *YopD* or the *YopD* chaperone (*LcrH*) failed to grow in

any rescue medium and are therefore truly temperature-sensitive."

"The Ca<sup>2+</sup>-blind yopD phenotype was fully suppressed in a Ca<sup>2+</sup>-independent background lacking the injectisome-associated inner-membrane component YscV but not peripheral YscK, suggesting that the core translocon energizes YopD."

The full article can be found at: (J.M. Fowler, et. al., "Growth of calcium-blind mutants of *Yersinia pestis* at 37 degrees C in permissive Ca<sup>2+</sup>-deficient environments." *Microbiology - SGM*, 2009;155(Part 8):2509-2521). Link not available.

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## **FINDINGS MAY LEAD TO NEW VACCINE STRATEGIES FOR PULMONARY TULAREMIA**

Medical News.net

October 23, 2009

"Immunologists at the University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh of UPMC and the have found a unique quirk in the way the immune system fends off bacteria called *Francisella tularensis*, which could lead to vaccines that are better able to prevent tularemia infection of the lungs. Their findings were published today in the early, online version of *Immunity*."

"*F. tularensis* is an intracellular pathogen that infects cells in the lungs called macrophages, explained senior author Shabaana A. Khader, Ph.D., assistant professor of pediatrics and immunology at the School of Medicine and an immunologist at Children's Hospital. Until now, scientists thought that eliciting a strong immune response to clear the infection would only require activation of a cytokine protein called interferon gamma (IFN-gamma). But that's not true for *F. tularensis* as it is for other intracellular bacteria, such as the TB-causing *Mycobacterium tuberculosis*.

"Our lab experiments show that in order to activate IFN-gamma in pulmonary tularemia, it is necessary to first induce production of another cytokine called interleukin-17," Dr. Khader explained. "So if we want to make an effective vaccine against tularemia, we must target ways to boost IL-17."

The full article can be found at: <http://www.news-medical.net/news/20091023/Findings-may-lead-to-new-vaccine-strategies-for-pulmonary-tularemia.aspx>

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## **A MODEL OF CARDIOVASCULAR DISEASE GIVING A PLAUSIBLE MECHANISM FOR THE EFFECT OF FRACTIONATED LOW-DOSE IONIZING RADIATION EXPOSURE**

By Mark P. Little\*, Anna Gola, Ioanna Tzoulaki

*PloS Computational Biology*

October 23, 2009

"Atherosclerosis is the main cause of coronary heart disease and stroke, the two major causes of death in developed society. There is emerging evidence of excess risk of cardiovascular disease in various occupationally exposed groups, exposed to fractionated radiation doses with small doses/fraction. The mechanisms for such effects of fractionated low-dose radiation exposures on cardiovascular disease are unclear. We outline a spatial reaction-diffusion model for early stage atherosclerotic lesion formation and perform a stability analysis, based on experimentally derived parameters. We show that following multiple small radiation doses the chemo-attractant (MCP-1) concentration increases proportionally to cumulative dose; this is driven by radiation-induced monocyte death. This will result in risk of atherosclerosis increasing approximately linearly with cumulative dose. This proposed mechanism would be testable. If true, it also has substantive implications for radiological protection, which at present does not take cardiovascular disease into account. The major uncertainty in assessing low-dose risk of cardiovascular disease is the shape of the dose response relationship, which is unclear in high dose data. Our analysis suggests that linear extrapolation would be appropriate."

The full article can be found at: <http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1000539;jsessionid=9C42E941D45C6DF31CD7A4E2F538F35B>  
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## **NEW EVIDENCE FOR TOXIC EFFECTS OF INHALED NANOTUBES**

By Hayley Birch  
Chemistry World  
October 25, 2009

“Further evidence for the asbestos-like effects of carbon nanotubes has emerged from a new study in mice. The study shows for the first time that the tubes reach the outer lining of the lung when inhaled - as asbestos does. But researchers say the results should be interpreted with caution.

Carbon nanotubes, like asbestos, have high aspect ratios; in other words, they are long and thin, meaning they have the potential to get stuck when trying to cross the two layered membrane - the pleura - separating the lung from the chest wall. In the case of asbestos, fibres can dwell in this area, causing lung disease and mesothelioma, a type of slow-growing cancer.

'We're not saying that carbon nanotubes are going to be like asbestos. We don't know yet,' says James Bonner of North Carolina State University in the US, who led the research. 'There's no evidence of cancer. The major finding is that we're saying that nanotubes get to the site where mesothelioma would occur, but we don't have the information to say that it does occur.'"

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“Ken Donaldson of the University of Edinburgh, one of the authors of the 2008 paper [C A Poland et al, *Nature Nanotechnology*, 2008, 3, 423 (DOI: 10.1038/nnano.2008.111)], stresses the importance of distinguishing between different types of nanotubes. 'My guess would be that the smallest ones are the least likely to cause much in the way of disease and that the longest ones would be most likely to cause disease,' he says. 'We're not in any position to be able to say this study has generic significance for all other nanotubes, because they come in different lengths, compositions and contaminants.'"

Bonner agrees, pointing out that the toxic effects could even be related to the nickel catalysts left over from nanotube growth - other manufacturing processes use different catalysts. He says further studies comparing the effects of nanotubes from different sources, of different sizes and at lower doses are required."

The full article can be found at: <http://www.rsc.org/chemistryworld/News/2009/October/25100901.asp>  
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