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Chem-Bio News – S&T Supplement

1. A DUAL-PURPOSE PROTEIN LIGAND FOR EFFECTIVE THERAPY AND SENSITIVE

DIAGNOSIS OF ANTHRAX: *"Finally, VWA-Fc is utilized as the capture molecule in the sensitive (down to 30 picomolar) detection of PA using surface plasmon resonance."*

2. AMPLIFICATION OF MICROSPHERE-BASED MICROARRAYS USING CATALYZED

REPORTER DEPOSITION: *"Furthermore, increases in variability resulted in poorer performance of TSA [tyramide signal amplification]-interrogated assays for botulinum toxoid, indicating that assay-specific optimization should be performed, especially prior to multiplexing."*

3. BUILDING THE BLOOD-BRAIN BARRIER [BBB]: *"This suggests that Wnt signaling might be tweaked to mend the BBBs in patients where it has failed—such as in stroke—or to temporarily open the BBB to deliver drugs that would normally be shut out." [emphasis added]*

4. "OLD BLOOD" LINKED TO INFECTION: *"Blood stored for 29 days or more, nearly two weeks less than the current standard for blood storage, is associated with a higher infection rate in patients who received transfusions with the blood."*

5. IMMUNOGENICITY OF BACILLUS ANTHRACIS PROTECTIVE ANTIGEN DOMAINS AND EFFICACY OF ELICITED ANTIBODY RESPONSES DEPEND ON HOST GENETIC

BACKGROUND: *"The results suggest that the variability observed in vaccination studies with PA-derived vaccines is a result of host heterogeneity and implies a need to develop other antigens as vaccine candidates."*

6. YERSINIA PESTIS TYPE III SECRETION SYSTEM-DEPENDENT INHIBITION OF

HUMAN POLYMORPHONUCLEAR LEUKOCYTE FUNCTION: *"In summary, our results suggest that the *Y. pestis* TTSS contributes to extracellular survival following interactions with human PMNs and that the intracellular fate is independent of TTSS inhibition of neutrophil ROS production."*

7. CASPASE-1 ACTIVATION IN MACROPHAGES INFECTED WITH YERSINIA PESTIS

KIM REQUIRES THE TYPE III SECRETION SYSTEM EFFECTOR YOPJ: *"This study*

uncovered a novel role for YopJ in the activation of caspase-1 in macrophages."

8. ORAL VACCINATION AGAINST BUBONIC PLAGUE USING A LIVE AVIRULENT

YERSINIA PSEUDOTUBERCULOSIS STRAIN: *"Our results thus validate the concept that an attenuated *Y. pseudotuberculosis* strain can be an efficient, inexpensive, safe, and easy-to-produce live vaccine for oral immunization against bubonic plague."*

9. DESIGN AND SYNTHESIS OF ARYL ETHER INHIBITORS OF THE REDUCTASE

BACILLUS ANTHRACIS ENOYL-ACP: *"X-ray crystal structures of BaENR in complex with triclosan and two other compounds help explain the improved efficacy of the new compounds and suggest future rounds of optimization that might be used to improve their potency."*

CB Daily Report

Chem-Bio News

A DUAL-PURPOSE PROTEIN LIGAND FOR EFFECTIVE THERAPY AND SENSITIVE DIAGNOSIS OF ANTHRAX

Drug Week

November 7, 2008

"This article reports the design of a bivalent protein ligand with dual use in therapy and diagnosis of anthrax caused by *Bacillus anthracis*. The ligand specifically binds to PA and thereby blocks the intracellular delivery of LF and EF toxins that, respectively, cause cell lysis and edema," scientists in the United States report (see also Anthrax Therapy).

"The ligand is a chimeric scaffold with two PA-binding domains (called VWA) linked to an IgG-Fc frame. Molecular modeling and binding measurements reveal that the VWA-Fc dimer binds to PA with high affinity ($K(D)=0.2$ nM). An in vitro bio-luminescence assay shows that VWA-Fc (at nanomolar concentration) protects mouse macrophages from lysis by PA/LF. In vivo studies demonstrate that VWA-Fc at low doses (approximately 50 microg/animal) are able to rescue animals from lethal doses of PA/LF and *B. anthracis* spores."

"Finally, VWA-Fc is utilized as the capture molecule in the sensitive (down to 30 picomolar) detection of PA using surface plasmon resonance."

The full article can be found at: (M. Vuyisich, et. al., "A dual-purpose protein ligand for effective therapy and sensitive diagnosis of anthrax". *The Protein Journal*, 2008;27(5):292-302). Link not available.

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AMPLIFICATION OF MICROSPHERE-BASED MICROARRAYS USING CATALYZED REPORTER DEPOSITION

"Assay sensitivities using three fluorescent signal generation schemes were evaluated on the Luminex flow cytometer. Following microsphere capture of antigen by immobilized antibodies, bound targets were quantified by use of (1) Cy3-labeled 'tracer' antibodies (30 min total time), (2) biotinylated tracers followed by streptavidin-R-phycoerythrin (60 min total time), or (3) biotinylated tracers followed by avidin-peroxidase conjugates and tyramide signal amplification (TSA; 90 min total time)."

"Use of TSA for signal generation in three individual toxin assays improved performance up to 100-fold over Cy3-antibody-based detection, and while streptavidin-R-phycoerythrin provided equivalent sensitivities, TSA produced dramatic increases at low concentrations simplifying positive sample identification. Detection limits for TSA-interrogated assays for ricin, cholera toxin, and staphylococcal enterotoxin B were 64 pg/ml, 4 pg/ml, and 0.1 ng/ml, respectively, using optimized conjugates; analogous detection limits for Cy3-antibody-interrogated assays were 8 ng/ml, 1 ng/ml, and 1 ng/ml, respectively. No improvement was observed in botulinum toxoid A assays when TSA amplification was used. As unique preferences for specific avidin-peroxidase conjugates were observed in the individual assays, improvements in multiplexed assays utilizing a single conjugate were significantly lower (3-10-fold improvements)."

"Furthermore, increases in variability resulted in poorer performance of TSA-interrogated assays for botulinum toxoid, indicating that assay-specific optimization should be performed, especially prior to multiplexing."

The full article can be found at: (G.P. Anderson, et. al., "Amplification of microsphere-based microarrays using catalyzed reporter deposition". *Biosensors and Bioelectronics*, 2008; 24 (2): 324-8). Link not available.

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BUILDING THE BLOOD-BRAIN BARRIER [BBB]

PhysOrg

October 27, 2008

"In brain endothelial cells, Wnt signaling was active during the time of maximum vascular development, but not after the BBB matured. Activation of the Wnt signaling pathway in vivo and in vitro promoted BBB development, and inactivation prevented it. In vitro increasing Wnt signaling also strengthened junctions between non-brain endothelial cells.

This suggests that Wnt signaling might be tweaked to mend the BBBs in patients where it has failed—such as in stroke—or to temporarily open the BBB to deliver drugs that would normally be shut out." [emphasis added]

The full article can be found at: <http://www.physorg.com/news144303683.html>

The original article can be found at: Liebner, S., et al. 2008. J. Cell Biol. doi:10.1083/jcb.200806024.

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“OLD BLOOD” LINKED TO INFECTION

Infection Control Today Magazine
October 28, 2008

“Blood stored for 29 days or more, nearly two weeks less than the current standard for blood storage, is associated with a higher infection rate in patients who received transfusions with the blood. In a new study presented at CHEST 2008, the 74th annual international scientific assembly of the American College of Chest Physicians (ACCP), researchers found that patients who received transfusions with blood stored for 29 days or more were twice as likely to suffer from nosocomial infections, including pneumonia, upper respiratory infections, and sepsis, with the oldest blood being associated with the most infections. Currently, federal regulations allow red blood cells to be stored up to 42 days, after which they must be discarded.

“Stored red blood cells undergo changes that promote the release of a number of biochemical substances called cytokines, which can depress the recipients’ immune function and leave them more susceptible to infection,” said study author Raquel Nahra, MD, who conducted her research while at Cooper University Hospital in Camden, N.J. “Those changes start around 14 days of storage and reach a maximum after the blood is discarded at 42 days.”

The full article can be found at: <http://www.infectioncontroltoday.com/hotnews/old-blood-linked-to-infection.html>

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IMMUNOGENICITY OF BACILLUS ANTHRACIS PROTECTIVE ANTIGEN DOMAINS AND EFFICACY OF ELICITED ANTIBODY RESPONSES DEPEND ON HOST GENETIC BACKGROUND

Medical Letter on the CDC & FDA
November 9, 2008

“.....vaccinated individuals demonstrate considerable variability in their antibody responses to PA. To explore the relationship between PA structure and antigenicity, we produced Escherichia coli strains expressing full-length PA (PA1-4), domains 2 to 4 (PA2-4), domain 1, (PA1), and domain 4 (PA4) and evaluated the immunogenicities and protective efficacies of the protein fractions in four mouse strains (strains A/J, BALB/c, C57BL/6, and Swiss Webster). Immunization with PA1-4 resulted in significantly higher lethal toxin-neutralizing antibody titers than immunization with any recombinant protein (rPA) fraction of PA. The magnitude and neutralizing capacity of the antibody response to full-length PA and its

fragments varied depending on the mouse strain. We found no correlation between the antibody titer and the neutralizing antibody titer for A/J and Swiss Webster mice. In C57BL/6 mice, antibody titers and neutralization capacity correlated for two of four rPA domain proteins tested, while BALB/c mice displayed a similar correlation with only one rPA. By correlating the reactivity of immune sera with solvent-exposed linear peptide segments of PA, we tentatively assign the presence of four new linear B-cell epitopes in PA amino acids 121 to 150, 143 to 158, 339 to 359, and 421 to 440. We conclude that the genetic background of the host determines the relative efficacy of the antitoxin response."

"The results suggest that the variability observed in vaccination studies with PA-derived vaccines is a result of host heterogeneity and implies a need to develop other antigens as vaccine candidates."

The full article can be found at: (N. Abboud, et. al., "Immunogenicity of Bacillus anthracis protective antigen domains and efficacy of elicited antibody responses depend on host genetic background". *Clinical and Vaccine Immunology*, 2008; 15(7): 1115-1123). Link not available.

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YERSINIA PESTIS TYPE III SECRETION SYSTEM-DEPENDENT INHIBITION OF HUMAN POLYMORPHONUCLEAR LEUKOCYTE FUNCTION

TB & Outbreaks Week
November 4, 2008

"Following phagocytosis by human PMNs, microorganisms are killed by reactive oxygen species (ROS) and microbicidal products contained within granules. *Yersinia pestis*, the causative agent of plague, is capable of rapid replication and dissemination from sites of infection in the host. Although *Y. pestis* survives in macrophages, the bacterial fate following interaction with human PMNs is less clear. The ability of *Y. pestis* to inhibit phagocytosis by human PMNs was assessed by differential fluorescence microscopy and was shown to be dependent on expression of the type III secretion system (TTSS). Previous studies have demonstrated that TTSS expression in enteropathogenic *Yersinia* spp. also inhibits the respiratory burst in PMNs and macrophages, and we show here that human PMN ROS production is similarly repressed by *Y. pestis*. However, exclusion of uningested TTSS-expressing *Y. pestis* with gentamicin revealed that intracellular bacteria are eliminated by human PMNs, similar to bacteria lacking the TTSS."

"In summary, our results suggest that the *Y. pestis* TTSS contributes to extracellular survival following interactions with human PMNs and that the intracellular fate is independent of TTSS inhibition of neutrophil ROS production."

The full article can be found at: (J.L. Spinner, et. al., "*Yersinia pestis* type III secretion system-dependent inhibition of human polymorphonuclear leukocyte function". *Infection and Immunity*, 2008; 76(8):3754-3760). Link not available.

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CASPASE-1 ACTIVATION IN MACROPHAGES INFECTED WITH YERSINIA PESTIS KIM REQUIRES THE TYPE III SECRETION SYSTEM EFFECTOR YOPJ

Medical Letter on the CDC & FDA

November 9, 2008

"Pathogenic *Yersinia* species utilize a type III secretion system (T3SS) to translocate effectors called *Yersinia* outer proteins (Yops) into infected host cells. Previous studies demonstrated a role for effector Yops in the inhibition of caspase-1-mediated cell death and secretion of interleukin-1 beta (IL-1 beta) in naive macrophages infected with *Yersinia enterocolitica*."

"Naive murine macrophages were infected with a panel of different *Yersinia pestis* and *Yersinia pseudotuberculosis* strains to determine whether Yops of these species inhibit caspase-1 activation. Cell death was measured by release of lactate dehydrogenase (LDH), and enzyme-linked immunosorbent assay for secreted IL-1 beta was used to measure caspase-1 activation. Surprisingly, isolates derived from the *Y. pestis* KIM strain (e. g., KIM5) displayed an unusual ability to activate caspase-1 and kill infected macrophages compared to other *Y. pestis* and *Y. pseudotuberculosis* strains tested. Secretion of IL-1 beta following KIM5 infection was reduced in caspase-1-deficient macrophages compared to wild-type macrophages. However, release of LDH was not reduced in caspase-1-deficient macrophages, indicating that cell death occurred independently of caspase-1. Analysis of KIM-derived strains defective for production of functional effector or translocator Yops indicated that translocation of catalytically active YopJ into macrophages was required for caspase-1 activation and cell death. Release of LDH and secretion of IL-1 beta were not reduced when actin polymerization was inhibited in KIM5-infected macrophages, indicating that extracellular bacteria translocating YopJ could trigger cell death and caspase-1 activation."

"This study uncovered a novel role for YopJ in the activation of caspase-1 in macrophages."

The full article can be found at: (S. Lilo, et. al., "Caspase-1 activation in macrophages infected with *Yersinia pestis* KIM requires the type III secretion system effector YopJ". *Infection and Immunity*, 2008;76(9):3911-3923). Link not available.

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ORAL VACCINATION AGAINST BUBONIC PLAGUE USING A LIVE AVIRULENT YERSINIA PSEUDOTUBERCULOSIS STRAIN

Biotech Week

November 5, 2008

"We evaluated the possibility of using *Yersinia pseudotuberculosis* as a live vaccine against plague because it shares high genetic identity with *Y. pestis* while being much less virulent,

genetically much more stable, and deliverable orally. A total of 41 *Y. pseudotuberculosis* strains were screened by PCR for the absence of the high pathogenicity island, the superantigens YPM, and the type IV pilus and the presence of the pYV virulence plasmid."

"One strain (IP32680) fulfilled these criteria. This strain was avirulent in mice upon intragastric or subcutaneous inoculation and persisted for 2 months in the mouse intestine without clinical signs of disease. IP32680 reached the mesenteric lymph nodes, spleen, and liver without causing major histological lesions and was cleared after 13 days. The antibodies produced in vaccinated animals recognized both *Y. pseudotuberculosis* and *Y. pestis* antigens efficiently. After a subcutaneous challenge with *Y. pestis* CO92, bacteria were found in low amounts in the organs and rarely in the blood of vaccinated animals. One oral IP32680 inoculation protected 75% of the mice, and two inoculations induced much higher antibody titers and protected 88% of the mice."

"Our results thus validate the concept that an attenuated *Y. pseudotuberculosis* strain can be an efficient, inexpensive, safe, and easy-to-produce live vaccine for oral immunization against bubonic plague."

The full article can be found at: (Oral vaccination against bubonic plague using a live avirulent *Yersinia pseudotuberculosis* strain. *Infection and Immunity*, 2008;76(8):3808-3816).

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DESIGN AND SYNTHESIS OF ARYL ETHER INHIBITORS OF THE REDUCTASE BACILLUS ANTHRACIS ENOYL-ACP

Blood Weekly

November 6, 2008

The problem of increasing bacterial resistance to the current generation of antibiotics is well documented. Known resistant pathogens such as methicillin-resistant *Staphylococcus aureus* are becoming more prevalent, while the potential exists for developing drug-resistant pathogens for use as bioweapons, such as *Bacillus anthracis*."

"The biphenyl ether antibacterial agent triclosan, exhibits broad-spectrum activity by targeting the fatty acid biosynthetic pathway through inhibition of enoyl-acyl carrier protein reductase (ENR) and provides a potential scaffold for the development of new, broad-spectrum antibiotics. We used a structure-based approach to develop novel aryl ether analogues of triclosan that target ENR, the product of the *fabI* gene, from *B. anthracis* (BaENR). Structure-based design methods were used for the expansion of the compound series including X-ray crystal structure determination, molecular docking, and QSAR methods. Structural modifications were made to both phenyl rings of the 2-phenoxyphenyl core. A number of compounds exhibited improved potency against BaENR and increased efficacy against both the Sterne strain of *B. anthracis* and the methicillin-resistant strain of *S. aureus*."

"X-ray crystal structures of BaENR in complex with triclosan and two other compounds help

explain the improved efficacy of the new compounds and suggest future rounds of optimization that might be used to improve their potency."

The full article can be found at: (S.K. Tipparaju, et. al., "Design and synthesis of aryl ether inhibitors of the reductase Bacillus anthracis enoyl-ACP". Chemmedchem, 2008;3(8):1250-1268). Link not available.

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