Unconventional Means To Combating Infections Gain Credibility, Interest

Jeffrey L. Fox

“A few years ago, a session on this subject couldn’t draw flies,” said symposium co-convener Steven Projan of MedImmune in Gaithersburg, Md., to a packed audience. His comments came at the start of the symposium, “Alternative Treatment Approaches to Bacterial Infections,” of the 2014 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in Washington, D.C., last September.

One such alternative approach to antibacterial agents involves the use of specific pathogen-targeted monoclonal antibodies (mAbs)—in general, to augment rather than outright replace conventional antibiotics, according to one of the symposium participants, C. Ken Stover, also from MedImmune. To overcome earlier failures when trying to harness mAbs into being effective agents against bacterial pathogens, Stover and his collaborators shifted strategies to take what he calls a “multifunctional approach.” Taking cues from Pseudomonas aeruginosa being “versatile and opportunistic,” that strategy entailed identifying mAbs “to several targets” on that bacterium, he says.

The development of “bi-specific” mAbs, which target two sites on that pathogen yields a product that is better than are the two single-acting mAbs used together to protect animals against P. aeruginosa, Stover continues. “We’re not sure why.” That bi-specific monoclonal was slated to begin phase 1 clinical trials “any day now,” he noted in September. With that mAb plus another mAb that targets Staphylococcus aureus, longer-term plans call for using these or other mAbs either prophylactically or in conjunction with conventional antibiotics to treat ongoing infections. Precepts include “never treat with [mAbs] alone,” and “we hope to preserve antibiotics for the longer haul,” he says.

Another approach to curbing the damages inflicted during infections with P. aeruginosa begins with a “focus on inhibiting virulence through the target of quorum sensing (QS),” says Laurence Rahme of Massachusetts General Hospital and Harvard Medical School in Boston, Mass., another symposium participant. These bacteria have three QS systems, one of which controls dozens of small molecules that contribute to virulence, she says. She and her collaborators recently identified a series of benzamide-benzimidazole molecules that prevent the synthesis of these virulence factors—in effect, declawing this bacterial pathogen. Another set of molecules shuts down yet other virulence functions. “These inhibitors can work against chronic and acute and multidrug-resistant strains,” she says. “And we’ve not seen the development of resistance. I’m not saying this will never happen, but clinical isolates don’t have [resistance-conferring] mutations.”

“Humans are perfused with antimicrobial peptides, and some are optimized to particular [anatomic] niches,” says symposium participant Michael Yeaman of Harbor-UCLA Medical Center in Torrance, Calif. Despite efforts to develop some of them as antimicrobial drugs, their “pharmacology is poorly understood” and several otherwise promising candidates failed to gain regulatory approval when reviewed by Food and Drug Administration (FDA) officials.

This outlook could be changing, particularly as the importance of using particular peptides “at sites for which...”

Color-enhanced transmission electron micrograph of negatively stained Pseudomonas aeruginosa. P. aeruginosa is the focus of recent research aimed at using specific pathogen-targeted monoclonal antibodies as part of efforts to treat infections. (Image © Kwangshin Kim/Science Source.)
they were evolutionarily designed” is taken into account, Yeaman says. “It’s not just the molecule, but when and where they may be used.” When those issues were taken into account, one such engineered kinocidin peptide, designated γ–RP-1, proved more effective than a conventional antibiotic when tested in mice infected with multidrug-resistant strains of Acinetobacter baumannii.

Yet another alternative approach calls for using bacterial cell wall-targeted lysin enzymes from bacteriophages as a means for combating bacterial pathogens, according to Vincent Fischetti from Rockefeller University in New York, N.Y. “Lysins have profound effects on biofilms,” he says. “They are effective against drug-resistant bacteria, work synergistically with antibiotics, are new agents against gram-positive bacteria, are safe, and it’s difficult to develop resistance to them.” These agents “work in animals,” he adds, and “clinical trials are expected soon.”

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2014 ICAAC

DNA Inhibitors, New Cephalosporins among Antibacterial Prospects

Jeffrey L. Fox

A potent new antimicrobial candidate drug that shares enzyme targets—but has alternate binding sites—with fluoroquinolones was featured during the poster summary session “Early New Antimicrobial Agents” at the 2014 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in Washington, D.C., last September. Other promising drug candidates are several novel cephalosporins, including one that carries a siderophore, a β-lactamase inhibitor that helps to overcome resistance in pathogens to β-lactam antibiotics, and an antifungal agent with an unusual mechanism.

Dual targeting of bacterial topoisomerase ATPase activity is a critical component of VXc-486, a “truly novel” aminobenzimidazole antibacterial agent, according to Susan Stokes of Vertex Pharmaceuticals in Boston, Mass. Although VXc-486 targets the bacterial topoisomerase enzyme—much like fluoroquinolone (FQ) antibiotics—its binding sites are different, and “we see no cross-resistance [with FQ antibiotics], and none is expected,” she says. This new antibacterial agent has a “broad spectrum,” with activities against many gram-positive and “some” gram-negative pathogens. The agent, which needs to be administered as a pro-drug to gain solubility in water, is bactericidal against pathogens such as Neisseria gonorrhoeae and Mycobacterium tuberculosis that are resistant to many kinds of antibiotics, giving it good “potential for multiple applications,” she says. When tested in monkeys, it shows little toxicity.

S-649266 is a novel, catechol-substituted siderophore cephalosporin that is active against gram-negative bacterial pathogens, including multidrug-resistant isolates of Pseudomonas aeruginosa and Acinetobacter baumannii, according to Yoshinori Yamano of Shionogi & Co., Ltd. in Osaka, Japan. The siderophore provides this candidate drug with an efficient means for entering bacterial cells via iron-transport mechanisms, he says. It is “highly stable” against various types of carbapenemases and β-lactamases. During phase 1 clinical studies, it was “well tolerated,” and there were “no significant adverse effects,” he adds. This cephalosporin is excreted via the urinary tract.

TD-1607 is a novel, “dual-mechanism,” heterodimer, glycopeptide-containing cephalosporin, one that is active against a variety of gram-positive bacterial pathogens, according to Edmund Moran of Theravance in South San Francisco, Calif. The glycopeptide portion enables the compound to bind to D-Ala-D-Ala side chains along the bacterial cell wall, placing the cephalosporin portion of the molecule next to penicillin-binding proteins, he says. It is “potent” when used as a single agent, its linker is chemically “stable,” and the intact molecule is 100-fold more active than are its two components separated but administered simultaneously, he adds. In phase 1 clinical studies, “all doses were well-tolerated, and there were no serious adverse effects.” TD-1607 is administered as an intravenous infusion, and it is excreted (mainly intact) through the urinary tract. Food and Drug Administration officials granted it “fast-track” status.

AA139, derived from the peptide Arenicin-3, shows potent bactericidal
MINITOPIC

One Extra-Long, Another Varied-Length Bacterium Manage To Split Evenly

Despite being varied in length in one case and extraordinarily long in the other, two different Gammaproteobacteria that live attached to the nematode worms Eubostrichus fertilis and E. dianae, respectively, divide symmetrically, according to Silvia Bulgheresi of the University of Vienna in Vienna, Austria, and her collaborators. The crescent-shaped bacterial symbiont associated with E. fertilis can vary as much as 12 times in its cell length, exceeding size variations seen in many more familiar bacteria, but nonetheless divides evenly into symmetrically sized daughter cells. In like fashion, the bacteria associated with E. dianae, which can grow to lengths of 120 μm, also neatly divides into equal-sized daughter cells—making it the “longest unicellular organism in which symmetric division has ever been observed,” the researchers note. These findings indicate that “size is not the primary trigger of division,” suggesting instead that “novel molecular machineries may time cell division and position the genome and division plane” in these bacteria. Details appeared 15 September 2014 in Nature Communications (doi:10.1038/ncomms5803).

activity specifically against gram-negative bacterial pathogens, according to Sergio Lociuro of Adenium Biotech in Copenhagen, Denmark. It appears to have a “dual mechanism of action,” targeting protein components of the outer membrane of such bacteria, but not lipid A, he says, adding: “The mechanism looks to be more complicated. It seems we’re working with multiple targets.” AA139 is active in treating several types of gram-negative infections in rodents, and shows “low or no toxicity,” as well as a “low propensity for resistance” to develop against it.

A diazabicyclic compound, designated OP0595, is a novel serine-β-lactamase inhibitor that acts mainly against A, B, and C-type β-lactamase enzymes, thus enhancing the activity of β-lactam antibiotics, according to Kenichiro Kondo of Meiji Seika Pharma Co. Ltd. in Tokyo, Japan. In terms of its safety, there are “no particular concerns,” he says. “We are moving forward” to develop this compound.

Finally, a novel natural product hexapeptide antifungal agent, designated ASP2397, is fungicidal against Aspergillus spp., is “highly potent” when evaluated in animals with azole-refractory aspergillosis, and leads to 100% survival in late-treatment models of such infections, says Ikuo Nakamura of Astellas Pharma in Tsukuba, Japan. ASP2397, which chelates iron and aluminum, is “actively taken up” by fungal cells, but “we haven’t identified the intracellular target within Aspergillus,” she adds. It has a “unique mechanism,” and is active against the conidial form of fungal pathogens, yielding a 3-log reduction within 8 hours.

2014 ICAAC

Systems Biology Using Host Responses To Diagnose Infections

Shannon Weiman

Researchers are taking a systems biology approach to identify pathogens—diagnosing diseases by analyzing specific responses that those pathogens elicit from hosts that they infect. Gene expression signatures, particularly in immune response pathways in blood samples, point to the cause of infection, according to several researchers who spoke during the symposium, “Systems Biological Approaches to Understanding Immunity in Vaccination and Infections in Humans,” convened as part of the 2014 ICAAC. These gene-expression signatures also help to predict responses to vaccines and may lead to speedier diagnoses and better patient outcomes.

“Different classes of pathogens trigger specific pattern-recognition receptors (PRRs) on peripheral blood leukocytes...inducing distinct gene expression profiles,” says Octavio Ramilo of Nationwide Children’s Hospital in Columbus, Ohio. These profiles serve as “disease fingerprints” for HIV, influenza virus, malaria, salmonella, and many other infectious agents, he says. In proof-of-concept studies, these profiles are impressively accurate in challenging circumstances, distinguishing among many different microbial causes of fever in infants with 90% sensitivity and specificity. In those cases where the patterns appeared to misdiagnose the pathogen causing symptoms, many of the patients developed the predicted infection weeks later, he adds.

Separately, Michael Levin of Imperial College in London, England, followed a similar approach to develop a point-of-care diagnostic test for tuberculosis (TB), which can be difficult to distinguish from other mycobacterial infections in patients, he says. However, a 44-transcript signature identifies Mycobacterium tuberculosis infections with 93% sensitivity and 88% specificity, he says.

“Functional fingerprints”—sets of transcripts indicative of responses by subsets of immune cells—can reveal pathogenesis patterns that are peculiar to specific pathogens, according to Ramilo. For example, the respiratory syncytial virus (RSV) suppresses B-cell responses, preventing them from producing protective antibodies. Vaccines to protect against this virus ought to take this suppression into account, he suggests. These patterns also provide information about individual outcomes. For example, the extent to
which RSV impairs immune responses significantly correlates with disease severity and length of hospitalization, he says.

Functional fingerprints show that M. tuberculosis suppresses T-cell receptor signaling and interferon production, according to Levin. “TB causes temporary acquired immune deficiency of the precise mechanisms required to contain the pathogen,” he says. For example, stimulating toll-like receptor 5 improves overall host responses to both influenza and polio vaccines, he adds, suggesting yet another approach for fine-tuning vaccines.

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RESEARCH ADVANCES

Some Cyanobacteria Acclimate To Using Far-Red Light for Photosynthesis

Barry E. DiGregorio

The cyanobacterium Leptolyngbya sp. strain JSC-1, isolated from a microbial mat from La Duke Hot Springs in Montana, not only can grow under far-red light but its photosynthetic apparatus also becomes more efficient at harvesting light energy under these ordinarily unproductive conditions, according to a team of microbiologists led by Donald Bryant of The Pennsylvania State University in University Park and collaborators from the University of California at Davis. They call this phenomenon far-red light photo-acclimation (FaRLiP) and speculate that it is widespread globally and important in environments enriched in such light. Details appear in the 12 September 2014 Science (doi10.1126/science.1256963).

When shifted from growth under white to far-red light, gene expression involving about 40% of the genome changes by more than twofold, with about 900 genes upregulated and another 2,000 downregulated, according to Bryant and his collaborators. The cyanobacteria replace 17 proteins in three major light-using complexes while also making two new chlorophyll pigments that can capture the far-red light. The cells also use accessory pigments called bilins in new ways. A 21-gene cluster, which includes a phytochrome that is responsive to far-red light and also two response regulators, appears to control expression of some of those several thousand genes.

Leptolyngbya sp. strain JSC-1 “changes the core components of the three major photosynthetic complexes,
so one ends up with a very differentiated cell that is then capable of growing in far-red light,” Bryant says. “The organism is better than other cyanobacterial strains at producing oxygen in far-red light and, in fact, it is even better than the same cells grown under other light conditions. Cells grown in far-red light produce 40% more oxygen when assayed in far-red light than cells grown in red light.”

Bryant and his collaborators recently identified five other strains that, like strain JSC-1, are also capable of FaRLiP. “A few are soil organisms and some others come from hot spring mats as well,” he says. “FaRLiP is likely to be driving a lot of terrestrial photosynthesis that otherwise would not be happening because of poor light penetration. All of the organisms to date that can do this also fix nitrogen. . . . We think this will be a globally important process.”

“The retention of a set of paralogous genes for the three major light-utilizing complexes in the genome, their differential expression, and biosynthesis of the complexes illustrates the extent to which photosynthetic organisms will go to capture solar photons and compete for photochemically active radiation,” says Charles Dismukes from Rutgers University of New Brunswick, N. J., referring to genes encoding proteins with similar function that likely arose via gene duplications. “This work highlights new possibilities that may be applicable to terrestrial crops to achieve improved performance.”

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NEW IN ASM JOURNALS

Microfluidics-Based Assay Identifies, Quantifies Viruses in Water Samples

David C. Holzman

By taking a microfluidics-based approach, a PCR assay was recently retooled for use in identifying and quantifying viruses in water samples, according to Satoshi Ishii of Hokkaido University in Sapporo, Japan, and his collaborators. “If we can quantify viral pathogens in hours, we can stop water distribution and disinfect drinking water before disease outbreaks occur,” he says. Details appeared 26 September 2014 in Applied and Environmental Microbiology (doi: 10.1128/AEM.02578-14).

Traditionally, water treatment utilities and food companies rely on measurements of fecal or total bacteria to assess the presence and abundance of enteric pathogens contaminating food and drinking water supplies. However, Ishii says, these measurements sometimes miss other important pathogens, including viruses, and thus can prove unreliable. Although he and his collaborators first applied their technology to measuring bacterial pathogens, all along they planned to adapt it to measure viral pathogens, he says.

“We performed multiple quantitative PCR in parallel, in nanoliter-volume chambers that are present in high densities on a chip,” Ishii continues. Although the technology was developed by other groups and is commercially available, this is the first report of its being used to detect and quantify several viral pathogens at once, he says. Other methods for simultaneously detecting several microbial pathogens do not provide quantitative information, or are slow and not particularly
NEW IN ASM JOURNALS

Enterotoxigenic E. coli Worldwide Are Closely Related: Portent of Success for Vaccine Development

Enterotoxigenic E. coli (ETEC) infect 400 million people annually, or 5.3% of the world’s population, killing 400,000. While ETEC were thought to vary widely from place to place, Åsa Sjöling, now of the Karolinska Institutet, Stockholm, Sweden, and collaborators of the University of Gothenburg, Sweden, and the Sanger Institute, Cambridge, UK, find that the two most potent toxins, LT1 and LT2, have changed little, but spread globally over the 30 years for which the investigators have isolates. That, says Sjöling, bodes well for a vaccine developed by the University of Gothenburg (where Sjöling did this work), which he expects “will be protective and useful globally since this vaccine is based on the toxin types and colonization factors we found to be most successful worldwide.”


NEW IN ASM JOURNALS

Salivary Mucins Play Active Role To Fight Cavities

A variety of mucins have been shown protective against certain pathogens, and defects therein have correlated with conditions such as asthma, and ulcerative colitis. Now Erica Shapiro Frenkel of Harvard University and Katharina Ribbeck of Massachusetts Institute of Technology find that salivary mucins, key components of mucus, actively protect the teeth from the cariogenic bacterium Streptococcus mutans. S. mutans attaches to teeth using sticky polymers that it produces, eventually forming a biofilm. “We found that salivary mucins don’t alter S. mutans’ growth or lead to bacterial killing over 24 hours,” says Frenkel. “Instead, they limit biofilm formation by keeping S. mutans suspended in the liquid medium. This is particularly significant for S. mutans because it only causes cavities when it is attached, or in a biofilm on the tooth’s surface.” The research suggests that bolstering native defenses might be a better way to fight dental caries than relying on exogenous materials, such as sealants and fluoride treatment, says Frenkel. Rather than simply a catchall filter for particles, “mucus is a sophisticated bioactive material with powerful abilities to manipulate microbial behavior,” says Ribbeck.

oughly address performance of RIDT for detecting influenza outbreaks. False-negative results are common, ranging from 40% in individuals with influenza A to 42–60% for influenza B. However, false positives are less than 1% for both influenza A and influenza B. “Health care providers should use positive influenza results obtained by RIDT to guide medical care with confidence,” says Peci. However, in individual testing, negatives should be followed by molecular tests such as reverse transcription polymerase chain reaction (RT-PCR), says Peci. Other findings include that RIDT detected influenza better in children and in the elderly than in nonelderly adults. The tests’ performance did not vary with the amount of circulating influenza viruses, indicating that it is a useful test at any time during, or even outside of the normal flu season. The team compared two forms of RIDT to RT-PCR.


NEW IN ASM JOURNALS

Mussels on California Coast Contaminated with *Giardia* Transmitted from Land-Based Sources

The pathogen *Giardia duodenalis* is present in mussels from freshwater runoff sites and from areas where California sea lions lounge along coastal California, according University of California, Davis investigators. One of the *G. duodenalis* strains found is known to infect humans; the two others occur mostly in dogs and other canids. These findings imply a “potential public health risk from fecally contaminated water or uncooked shellfish,” as they demonstrate that pathogens from land-based fauna are being washed into the sea, at least in the case of *Giardia*, where the same genotypes known to infect humans and canids were found in the mussels, says corresponding author Woutrina Smith. The question is still open in the case of *Cryptosporidium*, so oocysts with the correct appearance were detected in mussels via microscopy, but genotypes were not confirmed. Smith says the research reaffirms the usefulness of testing filter-feeding shellfish, that can process 2 liters of water per hour, thus concentrating pathogens in aquatic environments.


NEW IN ASM JOURNALS

Host Microbiota Influence Development: Another Example

Animals develop in the presence of complex microbial communities, and early host responses to these microbes can influence key aspects of development, such as maturation of the immune system, in ways that impact adult physiology. Ye Yang of the University of Oregon, Eugene, et al. previously showed that the zebrafish intestinal alkaline phosphatase (ALPI) gene alpi.1 was induced by gram-negative bacteria-derived lipopolysaccharide (LPS), a process dependent on myeloid differentiation primary response gene (88) (MYD88), and functioned to detoxify LPS and prevent excessive host inflammatory responses to commensal microbiota in the newly colonized intestine. Now they show that among the mouse ALPI genes, Akp3 is specifically upregulated by the microbiota, but through a mechanism independent of LPS or MYD88. “We showed that disruption of Akp3 did not significantly affect intestinal inflammatory responses to commensal microbiota or animal susceptibility to *Yersinia* pseudotuberculosis infection,” says Ye. “However, we found that Akp3<sup>−/−</sup> mice acquired LPS tolerance during post-weaning development, suggesting that Akp3 plays an important role in immune education. Finally, we demonstrated that inhibiting LPS sensing with a mutation in CD14 abrogated the accelerated weight gain in Akp3<sup>−/−</sup> mice receiving a high-fat diet, suggesting that the weight gain is caused by excessive LPS in Akp3<sup>−/−</sup> mice.”