Current Topics

Binding Agents Offer Strategy for Blocking Botulinum, Other Toxins

Delivering small molecules that bind botulinum toxin along with a monoclonal antibody (MAb) that binds them is being developed as a new strategy for rapidly and safely clearing this highly lethal toxin from the body, according to helminthologist Charles Shoemaker at the Tufts University Cummings School of Veterinary Medicine in North Grafton, Mass., and his collaborators there and at Thomas Jefferson University in Philadelphia, Pa. Their results from testing this strategy in mice appear in the February 2010 issue of *Infection and Immunity* (78:756–763).

Antidotes for biological toxins typically contain polyclonal antibodies, which are expensive to manufacture and stockpile, according to Shoemaker. In comparison, because his new approach is designed around specifically programmed, low-molecular-weight binding agents and a single, or universal, MAb, it offers a cheaper option with reduced overall development time, he says. The process could be used for making a variety of antitoxins with which to treat people exposed to one or more toxins as part of a bioterrorism attack or any from a broad range of natural toxins and also perhaps cytokines that the immune system releases during chronic illnesses.

To demonstrate the feasibility of their approach, Shoemaker and his collaborators derived single-chain Fv fragments (scFvs) from antibodies with a high affinity for serotype A botulinum toxin, which blocks transmission between nerves and muscles. The researchers tested six recombinant scFvs, each of which attaches to different sites along the botulinum toxin molecule. However, they also used a single anti-tag IgG1 MAb that recognizes all six scFvs.

Shoemaker and his team then tested which MAb-scFvs combinations could protect mice against botulinum toxin, administering doses that would be 1,000 to 10,000 times the 50% lethal dose. Untreated mice die within hours of receiving the toxin. However, mice treated by infusion with the MAb-scFvs mixture survive, and the toxin is excreted via the liver before it causes ill effects. Mice are not protected against botulinum toxin when they receive only scFvs or MAB without the other.

Although recombinant scFvs behaved well in these proof-of-principle experiments involving mice, alternative binding agents are being considered, including nanobodies and aptamers, according to Shoemaker. Since that study, they tried nanobodies as the binding agents and expressed different antitoxin nanobodies as a single protein agent. “With just a single agent, we can attract multiple MAbs to the toxin,” he says.

The Tufts researchers also are developing binding agents that target other types of botulinum toxin, Shiga toxin, and toxins from *Clostridium difficile*. Additionally, they plan to develop binding agents for clearing cytokines that play a part in causing inflammation responses that injure the host and in autoimmune diseases. Showing that the method is flexible and not limited to botulism “could make commercialization of the approach more appealing,” Shoemaker says.

“There’s strong value in this idea, and it looks like it can be generalized for a lot of different toxins or infectious agents,” says Kim Janda, a chemical biologist at Scripps Research Institute in La Jolla, Calif. He hopes that someone will evaluate whether this approach is safe for human patients. “It has all the right bells and whistles to make it worthy of clinical investigation,” he says.

Carol Potera
Carol Potera is a freelance writer in Great Falls, Mont.

Cyanobacteria from Earth Survive Rocky Ride through Space

A cyanobacterium species launched into low-earth orbit survived 10 days in an open container before being isolated in a terrestrial microbiology laboratory, according to microbiologist Karen Olsson-Francis of the Open University in Milton Keynes, United Kingdom, and her collaborators. Not only does this outlandish isolation procedure suggest that at least some bacteria could endure longer journeys through space, a deeper look into the fortitude of this cyanobacterial species might yield some tips as to what it takes to maintain extraterrestrial settlements with populations of plants, animals, and humans. Meanwhile, details of the microbial experiments appear in the April *Applied and Environmental Microbiology* (76:2115–2121).

The U.K. researchers isolated only a single species of bacterium from the terrestrial rock samples that contained an entire community when launched
and that traveled for 10 tough days in orbit about 300 km above Earth. That cyanobacterial species, designated OU-20, can also be isolated from limestone cliffs along the English Channel—the same source for the rocks that were launched into space, Olsson-Francis says.

The less-well-traveled samples were subjected to desiccation and vacuum in the laboratory before OU-20 was isolated, she points out. Based on both culture and culture-independent methods (16S rDNA), the U.K. researchers also found evidence of other microbial species, including Pleurocapsales, Oscillatoriales, and Chroococcales, in the same rock samples. In terms of morphology, OU-20 resembles members of the genus Gloeocapsa, which are found in biofilms colonizing granite in Antarctica.

“This is the first time that a novel organism has been isolated after exposure of a microbial community to outer space conditions,” says Gerda Horneck of the DLR German Aerospace Center, Institute of Aerospace Medicine. This is also the first investigation that used low-earth orbit to select for extremophiles, adds Olsson-Francis. The flight subjected the microbes to extremes of temperature, low pressure, cosmic radiation, and microgravity. The experimental container, called a Biopan, was closed before the orbiter landed to protect the experiments from the heat of re-entry.

The research was conducted with space applications in mind. The investigators chose the location where rocks were harvested, which is submerged in seawater at high tide, for its presumed diversity, in the hope of discovering novel, space-hardy species. Insights from these experiments could have applications for long-term space flights and extraterrestrial settlements in terms of determining requirements for “oxygen, fuel, and biomass production; nutrient acquisition, biomining; and feedstock provision,” according to Olsson-Francis and her collaborators.

These research findings confirm earlier observations that bacteria, cyanobacteria, and lichens survive short-term space flights, suggesting that “lithopanspermia may occur within a solar system,” says Rocco Mancinelli of the SETI Institute in Mountain View, Calif., referring to the hypothesis that life spreads through outer space when hardy seedlings travel on rocks from one celestial body to another.

The cyanobacteria likely survived their time in orbit in part because the rocks in which they were embedded protected against damage from ultraviolet (UV) light, Mancinelli says.
“The real factor affecting survival is extreme desiccation. This study seems to have selected for the organism that could best withstand desiccation in the space environment.” Olsson-Francis and her collaborators note that a thick, mucilaginous sheath, which can be observed via transmission electron microscopy, is also important for protecting dense colonies of OU-20 against UV light and other environmental insults.

The research confirms the value of exposing microorganisms to conditions in space exposure instead of depending on ground-based simulations, says Daniela Billi of the University of Rome, Italy. Adds Silvano Onofri of the University of Tuscia at Viterbo, Italy, these microbial findings “could modify the general perception of the central role of our planet in the origin of life.”

David Holzman
David Holzman is the Microbe Journal Highlights Editor.

**Outer-Membrane ATP Production: Another Surprise from *Ignicoccus***

The hyperthermophilic archaeon *Ignicoccus hospitalis* generates ATP on the inner side of its outer membrane and the energy molecules flow toward the inner membrane, according to Harald Huber at the University of Regensburg in Regensburg, Germany and his collaborators there and at the Johann Wolfgang Goethe University in Frankfurt. Furthermore, its unusual cellular architecture leads these researchers to speculate that if eukaryotic cells derived from an archaeal ancestor, as many believe, then *I. hospitalis*, or an organism much like it, is an ideal ancestral candidate. Details of this research appear in the February 16 *Proceedings of the National Academy of Science* (107:3152–3156).

“This most recent discovery is, to say the least, unexpected,” notes Moselio Schaechter of California State University at San Diego, “even for the hyperthermophile that shattered the ancient belief that life at high temperatures is not possible.”

All three known species in the crenarchaeal genus *Ignicoccus* lack rigid cell walls, specifically, a surface layer (S-layer) typical for other members of its phylum. Furthermore, also in contrast to other archaea, the inner and outer membranes of *Ignicoccus* enclose a compartment called the periplasmic space after the cell-wall containing periplasm of gram-negative bacteria. However, the archaeal outer membrane lacks lipopolysaccharide (LPS) and is porin-free, making it fundamentally different from that of gram-negative bacteria. Thus, *I. hospitalis*, along with *I. islandicus* and *I. pacificus*, have cell envelope architectures unique among archaea and unlike that of any known bacterium.

By examining the A₁ Aₙ ATP synthases of the *I. hospitalis* outer membrane, Huber and his team determined that this organism generates ATP within the periplasm, and that it flows inward towards the cytoplasmic membrane. In contrast, gram-negative bacteria generate ATP in their cytoplasmic membranes and transmit those molecules to the outer membrane. Because DNA and ribosomes are located in the cytoplasm of this archaeon, the researchers point out that ATP synthesis is spatially separated from information processing and protein biosynthesis.

Notably, says Huber, “Neither the inner nor the outer membrane of *I. hospitalis* alone satisfies all the criteria of a cytoplasmic membrane.” Although the outer membrane has a primary proton pump and contains ATP synthase, the inner membrane contains and surrounds the biochemical components necessary for information processing and biosynthesis. This arrangement raises the fundamental question of how to define cytoplasmic...

**Recent Deals, Progress toward Developing, Evaluating Antibiotics**

Several companies recently announced deals or financing to pursue development of novel antibiotics, including:

- In April, Pfizer of New York, N.Y., announced plans to collaborate with two biotechnology companies, MicuRx Pharmaceuticals of Union City, Calif., and Cumencor Pharmaceuticals in China, to develop antibiotics for drug-resistant tuberculosis in that country.
- Achaogen of San Francisco, Calif., secured $56 million in third-round financing, mainly from Frazier Healthcare Ventures, to fund a midstage trial of its candidate antibiotic for treating urinary tract infections.
- Cubist of Lexington, Mass., began enrolling patients for a phase II trial of CB-183,315, its candidate for treating *Clostridium difficile*-associated diarrhea.
- LFB Biotechnologies in Les Ulis, France, is collaborating with Thallion Pharmaceuticals in Montreal, Quebec, Canada, to develop Shigamabs, which is designed to treat infections caused by Shiga toxin-producing *Escherichia coli*; a phase II clinical trial is planned for later this year.
membranes generally, as well as in *Ignicoccus*.

Indeed, the cell structure of this archaeon is unlike that of any known bacterium but strikingly similar to that of eukaryotes. These differences make *I. hospitalis* a prime candidate for being a eukaryotic ancestor, one that could provide ATP and other molecules to an incorporated symbiont without the need for any interactions between the cytoplasm of either it or the host.

“This work also sheds light on the interaction between *I. hospitalis* (‘the friendly fire sphere’) and its companion archaeon, *Nanoarchaeum equitans* (‘the riding dwarf’), which has the smallest archaeal genome known and lacks nearly all metabolic and biosynthetic genes,” says Ulf Küper, the research team’s lead scientist. *N. equitans* grows exclusively on the surface of *I. hospitalis*, and the central question is how energy is transferred from host to rider. “Having ATP formation in the periplasm of *I. hospitalis* avoids the complex import of energy across three membranes into *N. equitans*,” Küper says.

The need for easy energy transfer from *I. hospitalis* to the nanoarchaeon may even have driven the evolution of its novel cellular architecture. “Archaea are full of surprises, as would be expected from organisms with such extreme survival talents,” says Schaechter, who believes that many more surprises are in store.

Marcia Stone
Marcia Stone is a science writer based in New York City. More of her work can be seen at http://www.mstoneworks.net.

Avatars in Games Help To Track Simulated Epidemics and Zoonoses

Analyzing disease outbreaks virtually—for example, by using popular gaming sites such as *World of Warcraft* (WOW) and *Whyville*—proves their value for estimating the dynamics of epidemics and for training specialists who might someday need to deal with real, not simulated, infectious diseases outbreaks. “Virtual outbreaks really do have some critical parallels with real-world outbreaks,” says Nina Fefferman, an epidemiologist at Rutgers University in New Brunswick, N.J., who spoke during the American Association for the Advancement of Science annual meeting, held in San Diego last March.

Infectious disease specialists began using computational modeling to track outbreaks years ago. For example, the Epidemiological Simulation System (EpiSims) (http://ndssl.vbi.vt.edu/episims.php) depends on a computer program to simulate smallpox outbreaks, and how vaccine campaigns and quarantines could help to curtail the spread of that virus.

One big problem with EpiSims and others like it is that they are programmed to follow normal behaviors of individuals, Fefferman says. “EpiSims wasn’t created to account for unusual behavior.” Although that model provides “very important insights,” it falls short in dealing with “unpredictability” and other situations for which there are “no good algorithms.”

However, some computer games are designed to let individuals react to such situations, according to Yasmin Kafai of the University of Pennsylvania Graduate School of Education in Philadelphia. “When we participate in a virtual epidemic outbreak, we experience... through our avatars what it’s like to get infected,” she says. “The opportunity to become ... an observer and a participant of how an epidemic plays out on multiple levels, the community, social interactions, and on your own decisions provides an unprecedented learning opportunity.”

Consider this scenario. Since 2001, the virtual community of Whyville experienced periodic outbreaks of WhyPox, a skin rash affecting participant avatars. During 2005, participants learned that isolating themselves from others who are infected avoids illness. During another outbreak, players...
Federal Court: Thimerosal in Vaccines Not a Cause of Autism

The U.S. Court of Federal Claims in Washington, D.C., ruled in three cases last March that thimerosal, a preservative used in vaccines, was not a cause of autism in children who received those vaccines. Those three cases were identified as representative prototypes for the approximately 5,000 unresolved cases in which parents filed lawsuits making claims against vaccines with mercury-containing thimerosal as a preservative. The rulings are considered part of the “largest omnibus proceeding in the history of the Vaccine Act,” a 1986 federal statute that establishes procedures for filing claims for alleged damages from vaccinations. “Having failed to satisfy their burden of proof under the articulated legal standard, petitioners cannot prevail on their claim of vaccine-related causation,” the court’s March ruling states. Details are available at http://www.uscfc.uscourts.gov/node/5026/.

could sell, trade, or donate a vaccine, whose use resonated more with girls, who sought preventive actions, than with boys.

Another game, WOW, becomes progressively more challenging, and players advance as their gaming skills improve. WOW programmers added an infectious disease outbreak called Corrupted Blood to an advanced level. Some players abandoned the disease level, moving their avatars back to less-complicated levels. However, in some cases, moving infected avatars to lower levels also spread the disease. Moreover, infected avatar pets also began spreading the disease throughout the levels, simulating a zoonosis.

In the cyber-world, epidemics spark panic, curiosity, altruism, and even mingling of infected and uninfected avatars, creating more chaos. “People are not always rational in their decision-making and behaviors,” Kafai says. “It’s perhaps not just the unpredictability that needs to be factored into models but also the ingenuity of unexpected behaviors that players display when online.”

“As not all epidemics are useful for epidemiology, not all epidemiological questions can or should be explored using virtual worlds,” Fefferman cautions. “I’m fond of the analogy of drug trials in mice: no one believes that a drug trial in a rodent will provide all the answers about the mechanisms and pathways and effects that a compound will cause in a human, but it’s a very good model system.”

Microbes in Cave Appear To Be Source of Rock Varnish

Some microorganisms not only coat rocks with a thin black veneer, they do so much faster than earlier estimates indicate, according to geomicrobiologist Michael Spilde from the Institute of Meteoritics at the University of New Mexico, Albuquerque, N.M., and his collaborators. Their results were presented last year during the Geological Society of America (GSA) annual meeting in Portland, Ore.

The microbes are actively making rock varnish in caves. Although no one claims to observe comparable biotic processes in desert settings, the varnish in caves appears the same as varnish found on desert rocks, suggesting it, too, has biologic origins. The humidity in caves may help to account for the relative speed of microbial varnish production there, according to Spilde. Based on manganese-oxidizing microbes that are actively producing rock varnish in Fort Stanton Cave in New Mexico, growth greatly exceeds the 40 μm per 1,000 years rate that others in this field consider standard.

Puzzlement over rock varnish dates back at least to naturalist Alexander von Humboldt. Considered the first geographer, he puzzled over the dark coatings that he observed on rocks and boulders while exploring South America during the early 1800s.

Two centuries later, geomicrobiologists Tanzhuo Liu and Wallace S. Broecker from the Lamont-Doherty Earth Observatory of Columbia University at Palisades, N.Y., did not say how rock varnish is made but concluded that it accumulates ever so slowly. Based on a set of 42 rock varnishes from western U.S. deserts, they reported in 2000 that that rock varnish grows at a rate of 1–40 μm per 1,000 years, making it the slowest known accumulating sedimentary deposit.

However, if Spilde and his collaborators are correct, cave varnish grows very quickly. For instance, in places where they removed samples a year ago, some regrowth has occurred. That comparative speed suggests that the availability of water or water vapor could accelerate growth of varnish in caves compared to rock varnish in desert settings.

“The walls are coated with black manganese oxide deposits that look so much like desert varnish that you would think you were on the desert surface instead of hundreds of meters underground,” Spilde says, referring to Fort Stanton Cave. “Some of the cave varnish is smooth and shiny just like varnish in the Mojave Desert, and contains micro-
laminations and microstromatolite structures.”

Those structures are consistent with microbial activity, Spilde continues. Several lines of evidence point to microbial origins for varnish, including: (i) laminated microstromatolite structure of manganese oxides, (ii) upward growth of structures toward the surface, (iii) manganese-oxidizing bacteria from the cave producing such materials in the lab, and (iv) DNA from the diverse suite of microorganisms from the cave varnish. “A few microorganisms are known manganese oxidizers, but a large percentage are novel,” he says. “We still at this point have many more questions.”

Geographer Ron Dorn of Arizona State University, an expert on rock varnish, is skeptical about comparing cave varnish with varnish on desert rocks, even though he suspects that the latter has biological origins. “I have no doubt that Spilde and his team have found rock varnish in the Fort Stanton Cave,” he says. “I do not think that they have proved the biogenic origin of rock varnish.” He also questions the legitimacy of “using a cave to ‘prove’ the biogenic formation of rock varnish that grows in the middle of a desert.” Adds another expert on rock varnish, Kim Kuhlman of the University of Wisconsin, “I personally don’t believe the cave coatings will ever be able to prove anything about varnishes outside in the desert simply because the conditions are so very different.”

Meanwhile, geologist David Krinsley from the University of Oregon examined several back-scattered electron microscope images from Spilde. “They look almost identical to the pictures I have taken of varnish in various places in the world,” Krinsley says. “I’d have to say that they are varnish.”

Barry E. DiGregorio
Barry E. DiGregorio is a freelance science writer in Middleport, N.Y.

Eyeing Data Torrents for Signs and Signatures of Pathogens

Some microbiologists relish each new firehose burst of data, holding their ground and expecting to learn something new and wondrous from each successive soaking. The optimists who adhere to this strategy declare that there surely will be something useful—a new pathogen, a sign of incipient infectious disease, perhaps even the signature of a bioterrorist attack—amid the torrents of potentially extraneous information. Maybe they will prove to be correct. For now, it was impressive hearing about some of their promising efforts during the 8th ASM Biodefense and Emerging Diseases Research Meeting, held in Baltimore, Md., last February.

One such approach entails “listening” to the host by monitoring gene expression patterns during infections, including at their earliest phases, according to David Relman of Stanford University in Palo Alto, Calif., who spoke during the plenary session, “Impacts of Genomics on Bioterrorism Preparedness.” Microarrays that look at gene transcript abundances provide a direct way to look at how hosts respond to diseases, including infections and cancer, he says.

However, the signs of infectious processes prove far more varied and “polymorphic” than do those even for cancer, making analysis “tougher,” Relman continues. An individual’s prior exposures to pathogens, which cells and tissues in the body are being examined, gender and age, time of day, and other factors contribute to the analytic complexity.

Nonetheless, in some cases, gene transcript patterns can prove useful for monitoring specific, infection-associated processes. For instance, although there is no “corroborated etiologic agent” for causing Kawasaki syndrome in children, this form of vasculitis yields a “distinct pattern” of transcripts, particularly among interferon response genes, when compared to other similar diseases affecting chil-

**Cryoadsorption Probe Detects Meat Spoilage at Early Stage**

A nondestructive analytic technique detects telltale low-volatile compounds, thus providing a rapid readout of whether chicken or other food products show early signs of spoilage, according to Tom Bruno and Tara Lovestead at the National Institute of Standards and Technology (NIST) in Gaithersburg, Md. Based on a probe that uses cryoadsorption to capture ordinarily poorly volatile ingredients, the NIST scientists identified six chemical markers that could be used to indicate poultry spoilage before the product becomes unsafe for consumers. Those markers appear in the air above samples of chicken meat that was maintained in its retail packaging material but kept at room temperature for two weeks. Details appear in the April 15, 2010 *Food Chemistry* (4:1274–1282). In a related development, Noam Sobel of the Weizmann Institute in Rehovot, Israel, and his collaborators are “training” comparable electronic odor-detection devices to predict and classify the relative pleasantness of novel odors. Their results indicate that odor perception is “hard-wired,” and could lead to new methods for odor screening, environmental monitoring, or perhaps transmitting scents digitally.
children, Relman says. That pattern might prove useful for recognizing ongoing Kawasaki and then guiding treatments with immunoglobulins, which prove critical for a fraction of children with that syndrome who are at risk for developing potentially lethal coronary aneurysms, he says.

About two years ago, W. Ian Lipkin of Columbia University in New York, N.Y., and his collaborators used genomic analysis to show there is no causal link between autism and use of the measles, mumps, and rubella vaccine (*Microbe*, November 2008, p. 508). What does give rise to autism remains an open question, he pointed out during another plenary session in February, “From Zoonosis to Pandemics.”

One noteworthy finding is that children with autism show “dramatic reductions” in enzymes that digest sugars, Lipkin continues. Moreover, there is a shift in the gut microflora of these children, including the appearance of a novel species of bacteria within that microbial community, and also evidence of damage along the intestinal surface that seems consistent with malabsorption of sugars among this group of children. If these observations hold up, they raise the possibility that autism could be either prevented or possibly corrected by countering such changes in the microflora of children with probiotics, antibiotics, or changes in diets, he points out.

Thomas Briese, who spoke in the same session as Relman and who is Lipkin’s colleague at Columbia, describes an approach to diagnosing infectious disease agents that depends on comprehensive DNA sequencing. Simply put, it consists of “sequencing everything, then looking at non-host segments and going from there,” he says. The key to this wholesale DNA-smashing approach is “pattern-recognition software” that will reliably pick up “uncharacteristic sequences”—in this case, those that arise from microbial pathogens of one sort or another. This approach proved critical several years ago, for example, for identifying a novel virus from the gastrointestinal tracts and brains of parrots, Briese says.

David Wang of Washington University School of Medicine in St. Louis, Mo., speaking during the same session, also favors a panoramic DNA sequencing approach, particularly to identify novel viruses as candidate pathogens. For instance, he and his collaborators recently identified several novel types of astroviruses from human stool specimens collected from patients with diarrhea in India.

Whether those viruses are truly pathogenic or mere bystanders remains to be determined. “There’s nothing to argue these are the bona fide [infectious disease] agents yet,” Wang says. Nonetheless, sequencing offers a powerful means for finding novel viral signatures, he adds. “DNA sequencing is more comprehensive and less biased than traditional methods.”

Jeffrey L. Fox
Jeffrey L. Fox is the *Microbe* Current Topics and Features Editor