Current Topics

After April Debut, H1N1 Influenza Virus Proves Deadly, Spreads Rapidly

Years spent anticipating the emergence of a pandemic influenza virus strain with the potential to spread readily among humans paid off in April when public health experts identified a novel “swine” influenza A (H1N1) virus as the source of a rapidly expanding and also deadly outbreak that centered and likely originated in Mexico. They quickly began tracking its spread into the United States, Canada, several countries in Europe, Israel, and New Zealand, while they also began implementing other measures intended to contain the damage this new virus might cause. Amid these fast-track responses at the national and international levels, however, the H1N1 flu epidemic continued to gather momentum after its springtime debut.

By late April, public health officials at the national, state, and local levels in the United States were rapidly gearing up to deal with focal swine flu outbreaks, which first hit mainly in California, New York City, and Texas, with some early cases involving travelers who recently returned from Mexico. By the end of April, officials at the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., reported nearly 100 laboratory-confirmed cases from 10 states, including one death, that of a 23-month-old child in Texas. More than half these early cases were among individuals less than 18 years old, according to CDC acting director Richard Besser.

Meanwhile, public health officials in Mexico were dealing with a proportionately larger and apparently more deadly epidemic on the basis of early statistics. For example, World Health Organization (WHO) officials reported that there were 1,626 confirmed human cases of infection, including 48 deaths in Mexico early in May. By then, WHO officials had documented nearly 4,700 total cases of H1N1 flu in 30 countries.

Within days of the first documented cases, U.S. federal officials and members of Congress were busily engaged in influenza-related activities, some with political overtones. For instance, political sensitivities doubtlessly contributed to an early renaming—replacing the initial “swine influenza” term with a more generic-sounding designation, the “2009 H1N1 influenza strain.” This U.S.-favored name change does not mean that the new flu strain suddenly dropped any of its porcine host-associated genes.

However, concerns that that having swine in the name would curb consumption of pork products and was already damaging international trade was very much in evidence during early congressional hearings and in statements from federal officials. Thus, for example, Agriculture Secretary Tom Vilsack in late April called U.S. pork products “safe” and said that there was “no evidence or reports that U.S. swine have been infected with this virus.” This message was intended not only for American consumers, but also for “trading partners” for whom “there is no basis for restricting imports of commercially produced U.S. pork and pork products,” he says.

Concerns over the expanding H1N1 flu outbreak likely also helped to finalize a Senate vote confirming Kathleen Sebelius, former governor of Kansas, to be Secretary of the federal Department of Health and Human Services. Within hours of being sworn in last April, she participated in briefings and led press conferences devoted to the H1N1 flu outbreak. She and other officials quickly announced steps to permit use of and distribute 50 million doses of antiviral products from the emergency federal stockpile, to distribute in vitro diagnostics for H1N1 flu, and to begin development of a seed stock for a vaccine. Experimental lots of a vaccine are expected to enter early clinical testing for safety, immunogenicity, and dosing within several months when other questions about scale-up and whether to formulate new H1N1 components with those for a seasonal flu vaccine will also be on the agenda.

Amid this frantic activity, federal experts were also trying to calibrate their response to channel public anxiety without creating panic. However, they also conveyed a sense that these efforts are not merely a drill. Calling the H1N1 influenza virus “prepandemic,” Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Md., notes that is “has never been seen before,” there is “no background immunity to it,” and it is “spreading from human to human.” Thus, this large-scale response is warranted, he and others say, expecting to make many adjustments as events unfold.

Jeffrey L. Fox
Antimicrobial-Producing Cells Part of Wound Dressing

Genetically engineering culture-grown human skin cells to produce an antimicrobial peptide makes them better at withstanding pathogens and could improve their overall clinical performance when used for dressing burns or other skin wounds, according to Lynn Allen-Hoffman at the University of Wisconsin-Madison (UW) and her collaborators at nearby Stratatech Corporation. “This is the first therapeutic use of cathelicidin,” she says, referring to the host defense peptide, whose gene hCAP-18 was inserted into and is expressed by the cultured cells.

The bacterial pathogen Acinetobacter baumannii provides some of the impetus for this line of research, according to Allen-Hoffman, a professor of pathology at UW. Multidrug-resistant isolates of A. baumannii from patients with surgical wounds, burns, or other skin injuries are increasingly common, prompting her interest in developing effective alternative means for treating them. That interest now is being focused on candidate products under development at Stratatech—the cathelicidin-producing skin cell line and a wound dressing that is made from those cells. A cathelicidin-free version of that product recently completed Phase 1/2 clinical trials in which it was used for covering wounds of burn patients.

Healthy human skin cells express hCAP-18, producing the broadly active antimicrobial peptide cathelicidin that kills gram-positive and gram-negative bacteria, fungi, and viruses. However, wounds, skin ulcers, and burn tissue either lack or do not express hCAP-18, making such sites more susceptible to infections. The UW and Stratatech researchers inserted “naked” DNA carrying the hCAP-18 gene into the skin cell line, thus circumventing viral gene transfer safety issues. The researchers chose to deliver the antimicrobial peptide directly through skin cells applied to wound sites because it could avoid any of the systemic effects associated with administering oral and intravenous antibiotics to patients, including opportunities for antibiotic resistance to develop.

When cultured skin carrying the hCAP-18 insert was exposed to the nonpathogen Staphylococcus carnosus, the defense peptide blocked bacterial growth by nearly 80% compared to the unmodified line of skin cells, according to Allen-Hoffman and her collaborators. They then tested whether the antimicrobial peptide-producing skin cells could protect mice with third-degree burns against a clinical isolate of A. baumannii that is resistant to 13 or more standard antibiotics. Within 72 hours of applying the cathelicidin-producing skin cells to the burn sites of the mice, pathogen numbers dropped 100- to 1,000-fold to levels that are no longer considered infectious. Details were reported online on 3 February in Molecular Therapy.

Encouraged by those findings, Allen-Hoffman and her collaborators are moving toward testing the cathelicidin-producing human skin cells in clinical trials. Recent discussions with officials of the Food and Drug Administration and members of the National Institutes of Health Recombinant DNA Advisory Committee proved encouraging, she says. “We were pleased with the outcome of those meetings and are moving forward.”

“The application of the transfected epithelial cells is obviously effective,” says Joost Oppenheim, chief of the Laboratory of Molecular Immunoregulation at the National Cancer Institute in Frederick, Md., referring to the in vitro and live-mouse experiments. However, he adds, simpler methods for delivering cathelicidin to skin wounds via gels, creams, or lotions should also be considered and might prove more practical.

Engineering the cultured human cells to produce cathelicidin, Allen-Hoffman says, enables them to deliver high levels of that antimicrobial to sites where it is most needed, she says. Moreover, it might not be appropriate to treat such wounds with ointments or gels. Further, if clinical testing proves successful, the synthetic skin-cell dressing could substitute for cadaver skin that, although used widely for treating burns and combat wounds, can cause problems for pa-

Host Factors in Hepatitis C Infections Seen as Novel Targets for Therapy

Nearly 100 host genes support the replication of the hepatitis C virus (HCV), and blocking several of them can suppress viral replication in cultured cells, according to Andrew Tai of Massachusetts General Hospital in Boston, Mass., and his collaborators. Thus some of these host factors might provide targets for novel therapeutic products, he says. The implicated host factors include a gene encoding the enzyme PI4KA that is involved in forming membranes within host cells where HCV replicates, another group of genes that coat vesicles and enable poliovirus to replicate, and the gene for hepcidin, a liver protein that regulates iron absorption. Blocking each of these genes blocks HCV replication. “This study is a proof of principle that targeting host factors is a viable therapeutic strategy,” he says. Details appear in the March 19 Cell Host & Microbe.
Microbes Engineered To Make High-Value Commodity Chemicals

With algorithms to help determine what genes to add or remove, bacteria can be engineered to produce industrial commodity chemicals from simple sugars such as glucose and sucrose, according to researchers at Genomatica, Inc. in San Diego, Calif. In February 2009, they announced the development of a microbially based process for making methyl ethyl ketone (MEK), which is used as a solvent for paints and varnishes.

“We’ve produced MEK from sucrose and glucose in two different microorganisms,” says Mark Burk, chief technology officer at the company. This environmentally friendly approach to producing MEK or other high-value commodity chemicals presents an alternative for making materials that ordinarily derive from crude oil. Moreover, it could lead to alternate uses for corn ethanol fermentation plants, many of which are underproducing or idle since oil prices dropped in the second half of 2008.

Last year, researchers at Genomatica engineered Escherichia coli cells to make the commodity chemical 1,4-butanediol (BDO), according to cofounder and president Christophe Schilling. A proprietary algorithm helped in identifying enzymes and corresponding genes in metabolic pathways that would be useful for synthesizing BDO from sucrose. That analysis led the researchers to move genes for eight particular enzymes into a strain of E. coli which was further modified by knocking out other genes, enabling it to continue producing BDO when media contain as much as 10% of that organic component. As part of this process, the modified bacterial cells are grown under anaerobic conditions.

The algorithms not only tell researchers what genes to add or knock out, but also how to tie “growth and survival of the microorganisms . . . to production of BDO,” Burk says. “The fastest-growing organisms are the ones that produce the most BDO.” He anticipates further improvements in yields, including by a scheme that calls for incorporating multiple metabolic pathways into BDO within a single E. coli strain. The company is designing a demonstration plant to scale-up production 1,000-fold. A key priority is to lower the cost of BDO, which now sells for about $1 per pound, according to Schilling. “We believe that we can make BDO substantially cheaper and advertise it as environmentally friendly,” he says.

“Just a few years ago, commodity chemical production in microbes was not realistic,” says Harvey Blanch, a chemical engineer at the University of California, Berkeley, and chief science technology officer at the Department of Energy’s Joint BioEnergy Institute in nearby Emeryville, Calif. However, advances in metabolic engineering and synthetic biology are changing that. BDO and MEK are good starting compounds to target for microbial synthesis because “they have much higher value than making ethanol from sugar,” he says. For example, annual global sales of MEK for use in paints and varnishes are about $2 billion.

“BDO is a good product to go after,” says Ryan Gill, a chemical and biological engineer at the University of Colorado, Boulder, who points to its worldwide annual production value of $4 billion, mainly as a feedstock for Spandex fibers and thermoplastics. Eventually, Genomatica could make their products more environmentally friendly by starting with cellulosic bio-mass instead of purified sugars, he points out. “It’s all a matter of economics, sustainability, and scale.”

Carol Potera

Studying Strep in Zebrafish Unveils Novel Virulence Factors

The gram-positive pathogen Streptococcus pyogenes carries genes encoding previously unidentified but likely virulence factors, including two macrolide efflux proteins, according to Anne E. Kizy and Melody N. Neely from Wayne State University School of Medicine in Detroit, Mich. These virulence factors were uncovered by studying what happens to zebrafish when infected by these bacteria, one among a variety of streptococci that sometimes cause necrotizing fasciitis, which in humans severely damages the skin. Details appear in the May Infection and Immunity (77:1854–1865).

“It took 6 months to determine the optimal infectious dose, the timing of infection, the tissue sites of analysis, the best way to grow the strains before inoculation, and the best way to determine strains missing after isolation of bacteria from the fish,” Neely says. As part of this project, she and Kizy assembled 1,200 mutant strains of S. pyogenes, a task that also took more than half a year. “This was a major undertaking, as Streptococcus can be a challenge to genetically manipulate,” she adds. The investigators screened those mutants for virulence genes via signature-tagged mutagenesis (STM), confirming that this approach could pick up known S. pyogenes-associated factors. “This was important since we were using a nonconventional model, the zebrafish, to look at disease caused by a human pathogen,” Neely says.

Zebrafish “have well-developed immune systems, including both innate and adaptive immunity, and many of
the same immune cells which are functionally and morphologically similar to those of humans,” Kizy says. Moreover, when the epidermal layer of zebrafish becomes infected with *S. pyogenes*, the damage and host response resemble what happens when humans develop necrotizing fasciitis. Thus, the “infection shows large aggregates of bacteria in the muscle tissue and a paucity of inflammatory cells at the site of infection—a typical phenotype for necrotizing fasciitis in humans,” she says. “We hypothesize that [the pathogens] are transporting a substance necessary for defending against the immune response. However, we do not yet know what each of these virulence factors do to the zebrafish.”

The newly identified virulence genes that encode macrolide efflux proteins do something other than to expel antibiotics from bacterial cells, according to Neely. “Our strain is not resistant to macrolide antibiotics,” she says. Moreover, says Kizy, “We concluded from the results of specific assays and PCR analysis that these efflux pumps are not directly involved in antibiotic resistance.” Because virulence of efflux pump knock-outs is “attenuated in this STN screen, we postulate that they possess some other inherent physiological role associated with virulence.” One of the two genes, *mfp*, “bears no homology to any known gene,” she says. Research to determine the virulence mechanisms of these genes is ongoing.

Further, this mutant set led them to identify another 24 genes that “are new to virulence, which we like to think means that they are important for necrotizing fasciitis,” says Neely. One of these, *amrA*, which other researchers had identified, influences optimal expression of *mga*, which in turn controls transcription of more than 10% of the *S. pyogenes* genome, “including not only the core regulon virulence genes, but also genes putatively involved in sugar metabolism,” Kizy says.

“The more we understand how group A streptococcus causes necrotizing fasciitis, the more likely we are to develop effective treatments for the disease,” says Nancy Freitag of the University of Illinois at Chicago College of Medicine, while praising Kizy and Neely’s approach. “This is a well-written and logically conceived paper that moves the field forward,” agrees Kevin McIver of the Maryland Pathogen Research Institute in College Park, Md.

“*S. pyogenes* is a major pathogen of humans, causing a wide array of diseases ranging from benign to life-threatening,” he continues. “Despite this, we still do not fully understand how a pathogen that typically causes strep throat can sometimes lead to severe invasive infections such as necrotizing fasciitis or streptococcal toxic shock syndrome. The results of this study provide new areas of focus for researchers to investigate that would not normally be followed in more traditional, single-gene-knockout approaches.”

David Holzman
David Holzman is the Microbe Journal Highlights Editor.

**Synthetic Coronavirus Is Chimeric SARS-Like Progenitor**

The severe acute respiratory syndrome (SARS) virus leaped onto the global stage in 2002 and, almost as abruptly, nearly vanished as a public health threat. During those few months on-stage, however, the SARS coronavirus proved responsible for about 800 deaths amid 8,000 infections—leaving plenty of questions about its origins, ecological habits, and lingering threats. One approach to probing those mysteries entails building, adapting, and analyzing synthetic versions of the SARS coronavirus and its likely progenitors, according to Ralph Baric of the University of North Carolina in Chapel Hill, who spoke during the plenary session, “Synthetic Genomes,” convened as part of the 7th ASM Biodefense & Emerging Diseases Research Meeting, held in Baltimore, Md., last February. This approach provides insights into the genetic changes needed for these viruses to shift host species and also into ways of atten-
Coronaviruses are one of several kinds of virus with a striking “history of host shifting,” Baric says. Sometimes that host shifting brings new diseases into humans, as was the case with SARS, while other times viruses move from humans into other animal species, as was the case with an epidemic in pigs several decades ago. Additional shiftiness of coronaviruses can take place within a species, when a virus moves from infecting one organ or organ tract to another, he adds. For instance, shipping fever in cattle, which causes severe respiratory distress, was once primarily an enteric disease.

In terms of host shifting for the SARS virus, the key molecular component that confers host versatility is its spike protein. Although about 3% sequence variation sets apart the human- and civet-infecting strains of the SARS virus in terms of the respective genes encoding this protein, those animal strains are mainly virtual, known merely in terms of sequence data, according to Baric. To tease out which changes in the spike protein are critical to host specificity and the shift from civets to humans, he and his collaborators generated a series of spike gene variants that they could “drop into” the cloned DNA version of the SARS virus genome. For safety reasons, the cloned viral genome was divided into a set of six fragments that could be efficiently consolidated, as needed and in controlled fashion, with the series of DNA fragments encoding those spike protein variants.

In one set of such experiments, the more human-like the spike protein, the better each synthetic viral strain grows in cultured human cells, and the more poorly it grows in cultured civet cells, according to Baric. When allowed to grow in particular types of cultured cells, the receptor binding domains of the spike protein “adapts rapidly,” to that cell type and the molecular contours of the receptor to which the virus binds on its way into those cells, he says.

Some of the synthetic viral variants behave very much like genuine SARS in animals, disrupting the lung epithelial layer and producing so much cellular debris that the infected animals experience acute respiratory distress, much like human patients with SARS. Further, and again much like SARS in humans, that distress was more pronounced in older than in younger animals. “SARS was more lethal in the aged population,” Baric points out. “It’s difficult to make a vaccine that targets this more susceptible population group.”

Although the SARS virus apparently moved from civets to humans at the outset of the 2002 pandemic, the horseshoe bat rather than the civet cat is considered the reservoir species for the progenitor virus, and dealing with the bat coronavirus is “more challenging” because of its greater “heterogeneity” and its higher frequency of recombinations, according to Baric. Yet, if SARS were to remerge in humans, this bat-associated “cluster” of coronaviruses rather than any isolated from civets or other animals is “what you would want to target,” he says.

So far, however, efforts to produce and study bat-like SARS progenitor viruses are full of frustrations. For one thing, he says, “no one has these viruses replicating in culture.” For another, there are many variations in the spike protein gene and also high error rates in the sequence data for this viral cluster. With so many moving molecular targets, he notes, “it’s easy to make dead viruses.”

However, it proved possible to produce a chimeric bat-human SARS-like virus—one that Baric calls the “biggest synthetic life form so far.” Although it is about four times larger than the other viruses made to date, this record “will probably be short-lived,” he says, alluding to ongoing efforts by a group at the J. Craig Venter Institute in Rockville, Md., to produce a synthetic bacterial cell.

More importantly, analysis of the
Phage Key to Assembling High-Performance Lithium Ion Batteries

When genetically engineered and in environmentally benign ways, bacteriophage can help to assemble appropriate materials into milligram-scale, high-power cathodes within lithium ion batteries, according to Angela M. Belcher of the Massachusetts Institute of Technology (MIT) in Cambridge, Mass., and her collaborators. Although too small yet for practical uses, the batteries have proportionately the same energy capacity and power performance as—and thus, in theory, could be made bigger to replace—the rechargeable batteries now used to power hybrid cars. For such reasons, the experimental batteries made a hit at the White House in early April when MIT president Susan Hockfield showed off the phage-based technology to President Obama and other members of the administration. Details of the research appear in the 2 April 2009 Science Express.

M13 filamentous bacteriophage particles are at the heart of these unusual batteries, whose power performance directly relates to how these viruses are used to assemble the batteries. Each 880-nm-by-6-nm phage particle carries an individual wire containing iron phosphate with a carbon nanotube at one end. These units are gathered en masse to constitute the cathode, one electrode of the battery. The other electrode, the anode, is also constructed with the virus but carrying gold-cobalt oxide (Microbe, July 2006, p. 309).

The overall performance has greatly improved since 2006, as has specific capacity, power density, energy density, rate capacity, and safety, according to Belcher. Of note, the cathode power performance is “particularly good,” she says. The batteries can store an electric charge until circuits are switched to enable this stored power to drive electronic devices. The carbon nanotubes are critical for moving that charge between the cathode and the circuit.

“The power performance of the battery is directly related to the affinity of the P3 protein for the carbon nanotube,” Belcher says, referring to a key structural protein of the phage capsid. For the process to work well, the researchers genetically modified P3, boosting its affinity for carbon nanotubes. Along with that increase in binding affinity, the power output of the device increases. The virus is also engineered to nucleate particles of iron phosphate, keeping them small and packing them closely, properties that contribute further to overall performance, according to Belcher. These
materials are fabricated environmentally benignly in aqueous buffers below 20°C, instead of at 350°C or higher in organic solvents, manufacturing conditions that are typical for conventional lithium ion batteries, she points out.

Although proteins and nucleic acids from the phage remain inside the batteries, those materials do not interfere with their operation, according to Mark Allen of MIT. “The protein is entirely coated by the iron phosphate mineral, with only the ends exposed to bind the nanotube,” he says. “Since the protein is never exposed, it wouldn’t interact negatively with anything. In this particular type of battery, the protein may not play any role after the mineral has been synthesized and assembled. Its main function is to act as a template on which to combine the carbon nanotubes with the iron phosphate mineral giving intimate contact between both.”

The phage-fabricated batteries are “durable,” according to Belcher. When operated through 100 charge-discharge cycles, they retain full capacitance, she says. However, she and her collaborators are tinkering with materials to improve performance and to scale up the devices for conventional use or for more exotic applications such as for carbon storage. For instance, they plan to substitute manganese phosphate for iron phosphate. Additionally, the batteries, now at milligram size, would need to be made in kilogram-sized packages to be used in automobiles or similar practical applications. At an intermediate size, they might be used to run personal electronic devices. They might also be used in other kinds of devices such as solar electricity cells, she says.

“The key contributions of this paper are making the iron phosphate nanomaterials and then assembling them with the carbon nanotubes,” says chemist Vince Rotello of the University of Massachusetts, Amherst. “Doing either individually using a phage display method is good; doing both simultaneously is impressive.” And while carbon nanotubes are fashioned under environmentally harsh conditions, “they simply provide a proof of principle for the controlled attachment of redox-active systems to synthetic materials,” he adds. “The method should be generalizable to other, greener connecting materials.” Nonetheless, much work would need to be done to put the technology into automobiles, because the revolutionary technology would require “huge investments” in retooling batteries. But he foresees even incrementally improved batteries being adopted for high value-added applications, for example, by the military sponsors of the work.

David Holzman

Assembling Genomes from Large DNA Segments In Vitro

Synthetic segments encompassing the 583-kbp genome of the bacterium *Mycoplasma genitalium* now can be fully assembled in vitro, avoiding dependence on yeast or other microbial cells for this final stage of assembly, according to Daniel Gibson and his collaborators at the J. Craig Venter Institute in Rockville, Md. The procedure entails mixing the large-scale DNA segments with three critical enzymes, one that partly digests the ends of DNA strands to create overlaps, another that fills those gaps, and a third that binds the ends of the DNA fragments to close those gaps. Removing yeast from this late-stage assembly step avoids any toxicity that such large fragments carrying diverse genes can cause when introduced into such cells, Gibson says. Details appear in the April 12 *Nature Methods*. 

**David Holzman**