Heart Drug Helps To Beat Chagas, Leishmania Parasites

Amiodarone, a drug long used for treating irregular heart rhythms, can also be effective against Chagas disease and leishmaniasis skin lesions, according to Alberto Paniz-Mondolfi at Columbia University in New York, N.Y., and his collaborators in Venezuela. Both these parasitic diseases are endemic in Central and South America, affecting millions. Moreover, cases in which patients are coinfect ed with these parasites are on the rise, the clinical researchers report.

Trypanosoma cruzi, a protozoan parasite that is transmitted by reduviid bugs, causes Chagas disease, which was discovered 100 years ago. During the chronic phase of an infection, these parasites can affect organs such as the heart, damaging muscle tissue and causing arrhythmias. Drugs that target this parasite show limited effectiveness, and toxic side effects restrict their use. Meanwhile, sand flies transmit Leishmania spp., another type of protozoan parasite. They cause leishmaniasis, which develops in several forms. For example, cutaneous leishmaniasis causes skin ulcers, whereas visceral leishmaniasis affects organs such as the liver and spleen. Here again, available therapy is unsatisfactory. For example, widely used pentavalent antimonials can damage the heart, kidneys, and liver. Thus, better drugs are needed for treating individuals infected with either or both these parasites. Vaccines are also needed.

A month after a Chagas patient received amiodarone for his heart arrhythmia, his levels of circulating antibodies against *T. cruzi* dropped dramatically, according to Paniz-Mondolfi. Subsequent treatment with the antifungal drug itraconazole lowered those levels below detectable limits. Details appear in the May 2009 issue of *Chemotherapy* (55:228–233).

Separately, amiodarone was given to a patient with cutaneous leishmaniasis to stabilize an irregular heart rhythm. Surprisingly, after a month, the leishmaniasis lesions healed without other treatments. Details of that case study appear in the June 2008 *Therapeutics and Clinical Risk Management* (4:659–663).

Since those cases, Paniz-Mondolfi and his colleagues successfully treated 12 more Chagas and leishmaniasis patients with amiodarone or amiodarone-itraconazole combination therapy, and are planning to conduct clinical trials on larger numbers of such patients.

When used against fungi, azole drugs block ergosterol biosynthesis and interfere with membrane biosynthesis. In treating heart arrhythmias, amiodarone disrupts mitochondrial calcium homeostasis. When combined to treat *T. cruzi* or *Leishmania mexicana*, the two drugs prove remarkably potent in killing the parasites. Details appear in the April 2009 *Antimicrobial Agents and Chemotherapy* (53:1403–1410) and the February 2006 *Journal of Medicinal Chemistry* (49:892–899).

“Chagas patients treated with ami-

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*T. cruzi* infecting heart tissue. *T. cruzi* nests in cardiac muscle with surrounding inflammatory cell reaction. Researchers find that drugs used to treat cardiac arrhythmia also have activity against these trypanosomes. (Micrograph courtesy of Alberto Paniz-Mondolfi.)
odarone for arrhythmias improved overall more than patients treated with other anti-arrhythmia drugs, and we wondered why,” Paniz-Mondolfi says. “These basic science results explain what we see clinically in patients.”

“It’s an excellent example of the piggy-back approach to the chemotherapy of tropical diseases,” says Roberto Docampo, a professor of cellular biology at the University of Georgia, Athens. The drug combination is attractive because both types of drugs are already approved for use in humans and much is known about their pharmacokinetics and side effects. Although long-term use of amiodarone for arrhythmia can be toxic, “its use in combination with imidazole to kill parasites could be shortened because of their synergistic effects,” he says.

Treating Chagas patients early with amiodarone and antifungal drugs of theazole type might eliminate the chronic phase that leads to heart problems, Paniz-Mondolfi says. More generally in terms of both Chagas and leishmaniasis, he adds, “In developing countries we need to provide patients with an immediate solution, and amiodarone and itraconazole are generic, cheap, and abundant.”

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

Low Phosphate Triggers Reversible Virulence in Pseudomonas

Phosphate shortages could explain how Pseudomonas aeruginosa shifts from being a mere colonizer of the human gastrointestinal (GI) tract into a lethal agent, says John Alverdy, a surgeon at the University of Chicago School of Medicine in Chicago, Ill. Although P. aeruginosa sometimes remains benign when it colonizes GI tracts, stresses such as surgery or illness often trigger virulence, potentially leading to inflammatory responses, sepsis, and death. However, adding phosphate can reverse that shift and protects animals against death from such infections, he and his collaborators report.

Invasive medical procedures such as radiation therapy, chemotherapy, transplants, and surgery “stir up Pseudomonas in the gut,” Alverdy says. To better understand this phenomenon, he and his collaborators introduced a benign strain of P. aeruginosa into the GI tracts of some mice but not others, and then surgically removed parts of their livers, an invasive procedure from which they ordinarily recover.

However, following surgery, the mice carrying that supposedly avirulent strain of bacteria developed severe complications, Alverdy says. “In response to the surgical stress, the bacterium switched its phenotype and somehow killed the mice.” Noting that phosphate levels drop drastically following major surgery, he decided to test whether phosphate affected the P. aeruginosa strain that the mice carried.

As part of their broader analysis, Alverdy and his collaborators switched host species, in part to simplify some of their manipulations, but also to determine whether the host contributes to this change in microbial virulence.

Preliminary Testing of the H1N1 Influenza Vaccine, Emergency Provisions for Diagnostics

The H1N1 influenza pandemic began to rebuild momentum throughout the Northern Hemisphere in September, and it was being met by a series of developments, including:

• Officials at the U.S. Food and Drug Administration (FDA) in mid-September approved four vaccines to protect against the H1N1 influenza virus, including injectable products from CSL Limited, Novartis Vaccines and Diagnostics Limited, and Sanofi Pasteur plus a live-attenuated, flu-virus product from MedImmune LLC that is administered intranasally.

• Results from phase-I clinical trials involving adults indicate that the injectable 2009 H1N1 influenza vaccines are well tolerated and induce strong immune responses when administered as single 15-μg doses, according to Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (NIH).

• Results from phase-I clinical trials with the H1N1 vaccines involving children indicate that those who are 10 and older develop an apparently protective response following a single injection, whereas children between 6 months and 9 years will likely require two doses of vaccine.

• Late in August, FDA issued an Emergency Use Authorization allowing the RT-PCR 2009 H1N1 influenza virus test to be used for detecting the virus among U.S. troops serving overseas.

ASM maintains a resource page on H1N1 flu that includes World Health Organization and Centers for Disease Control announcements and guidance documents. See the ASM website at http://www.asm.org/index.php?option=com_content&task=view&id=64294&Itemid=657
Thus, he and his team began studying the impact of P. aeruginosa on the worm Caenorhabditis elegans, which were fed diets containing the poorly virulent PAO1 strain of P. aeruginosa. The bacteria were grown in either high- or low-phosphate medium.

Worms that fed on P. aeruginosa from low-phosphate medium died after developing large red spots in the digestive tube, a novel syndrome that the researchers call “red death.” Adding phosphate or iron suppressed the red death. Meanwhile, those worms that were fed high-phosphate-grown P. aeruginosa thrived.

Alverdy and his collaborators next identified three virulence systems that appear to be activated in the Pseudomonas strain that grew on a low-phosphate medium and that caused red lesions in the worms. “A low-virulence strain of Pseudomonas can be turned into a potent killer simply by lowering phosphate,” he says. This shift from low to high virulence depends not only on phosphate depletion but also on iron signaling and quorum sensing. Details appear in the April 14, 2009, Proceedings of the National Academy of Sciences.

When the PAO1 strain is grown in media depleted of phosphate, the cells produce higher levels of the PA-I lectin and pyocyanin, form biofilms, and boost expression of PstS, a gene encoding a phosphate acquisition protein, as well as other genes whose products contribute to virulence. The PA-I lectin, for example, disrupts the epithelial barrier within the GI tract of mice, allowing exotoxin A to disseminate and cause sepsis. Biofilms interfere with antibiotics and host immune responses, while pyocyanin kills neutrophils.

These findings “highlight the importance of the nutritional environment in regulating virulence in Pseudomonas,” says Matt Parsek, a microbiologist at the University of Washington, Seattle. “It’s narrow-minded not to consider nutritional needs of bacterium,” yet few experiments consider how the host’s diet might affect microbial virulence factors. It is too early to say whether these findings will lead to a new therapeutic approach, he adds.

Nevertheless, Alverdy is already testing compounds that might prevent Pseudomonas from expressing virulence in the GI tracts of patients. One approach is to use polyethylene glycol polymers for delivering phosphate to the lower GI tract, as a way of overcoming its absorption in the upper GI and rapid loss in urine. His idea is that recovering surgical patients will drink this or some other phosphate-containing concoction to prevent bacteria from becoming virulent, and thus avoid a need for systemic antibiotics.

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

**Senate Bill Addresses Laboratory Biosecurity Issues**

Senators Joseph Lieberman (I-CT) and Susan Collins (R-ME) of the Homeland Security and Government Affairs Committee introduced legislation on 8 September aimed at strengthening security at high-containment biology laboratories. Provisions in the bill, the “Weapons of Mass Destruction Prevention and Preparedness Act of 2009,” charge the Department of Homeland Security (DHS) with determining which pathogens have the greatest potential for being used in terror attacks and also with implementing security standards for labs that study those pathogens, including by setting and enforcing personnel reliability standards. In a related development, Congress extended the mandate of the Weapons of Mass Destruction Commission. Its report, “World at Risk,” from December 2008 contains recommendations that helped to frame the Lieberman-Collins bill. Meanwhile, the Government Accountability Office (GAO) recommends in a September report (GAO-09–574), “High-Containment Laboratories: National Strategy for Oversight Is Needed,” that a new federal entity be established to oversee labs working on such pathogens.

**Better Satellite Surveillance Could Help Counter Disease Outbreaks**

“The earth is responding to escalating carbon dioxide levels with rising seas, flooding, droughts, forest fires, and a corresponding surge in infectious disease—some all too familiar; others new,” says Timothy Ford from the University of New England in Biddeford, Maine. “If properly done, predictive modeling will give us enough time to contain and possibly even prevent potential epidemic and pandemic diseases.”

“Even though satellite measurements and other remote sensing techniques can’t identify disease-causing pathogens and vectors directly, they can characterize the environments in which they thrive,” says Peter Gilruth, who directs the Division of Early Warning and Assessment at the United Nations Environment Programme. “Environmental variables, including land and sea surface temperatures as well as the amount, type, and health of vegetation, can be accurately identified with remote sensing techniques. Sensors are being used to collect information from space, while powerful algorithms and image-processing software on desktop computers make it feasible for biologists and epidemiologist to experiment with spatial analysis technology.”
In the United States, the National Aeronautics and Space Administration oversees much of the remote-sensing activities, while the National Oceanic and Atmospheric Administration operates valuable weather satellites. In addition, Canada, several European Union countries, Japan, and India have remote sensing satellites. While it would appear that the planet is being well watched, Ford decries “the crisis in U.S. funding that not only affects basic and applied research in this field but also undermines our ability to deploy sensing technologies that provide the most promising means of monitoring our environment.

“This is distressing because nothing is larger in scale, has more potential to cause long-term effects— and is more uncertain— than climate change when it comes to infectious disease outbreaks, epidemics, and pandemics,” Ford continues. He cites environmentally driven zoonotic and vector-borne infectious diseases as examples and adds “insect or rodent vectors make it virtually inevitable that pathogens will be globally transported by plane or boat.” More of Ford and colleagues’ views on this subject can be found in the September issue of Emerging Infectious Diseases (DOI: 10.3201/eid1509.081334).

When climate change and increasing populations triggered reemergence of Rift Valley fever (RVF) in Senegal, Africa, the French National Space Agency (CNES) used its geographical information system (GIS) and remote sensing (RS) imaging technology to detect potential breeding ponds for Culex poicilipes, an important vector mosquito, according to Antonio Güell at CNES. That information, combined with entomological data about the flying ranges and spatial distributions of mosquitoes, enabled the team to map high-risk RVF zones.

Remote sensing data have similar applications in North America. For example, assessing high-risk areas where insect vector-borne diseases such as Lyme disease, the West Nile virus, and dengue fever are likely to show up next. Notably, the mosquito Aedes albopictus, a native of Asia that serves as a vector for both the West Nile and dengue viruses, recently established itself in North America. “Yet, application of remote-sensing techniques to map areas at risk for dengue fever within this country has yet to be done,” Gilruth says.

“It should be emphasized that resource allocation for novel monitoring techniques should not come at the cost of basic disease prevention and management at the community level,” Gilruth continues. Nor should ground-based data collection and transmission networks be ignored (Microbe, March 2009, p. 108). These systems supported by satellite imagery are vital for the control of environmentally dependent diseases, Güell adds.

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.

Chirality Tests Offer Approach for Resolving Viking Mars Questions

If the Viking labeled-release experiment on Mars in 1976 had tested glucose optical isomers separately, it might have avoided lingering doubts about its apparently positive results suggesting biological activity, say microbiologist Henry J. Sun of the Desert Research Institute in Las Vegas, Nev., and his collaborators. Some scientists say that experiments at two different landing sites detected life, whereas others believe that chemical oxidants in soils merely mimicked microbial activity. However, in recent earthly simulations, microbial specimens consumed only D-glucose, not its L isomer, whereas chemical oxidants such as permanganate showed no preference between these two mirror-image sugars, they report in a recent issue of Astrobiology. Thus, they recommend conducting such a chirality-based experiment on a future Mars mission.

Of course, adapting this kind of experimental procedure for transport to...
Mars or some other planet will be complicated and challenging. For example, how can it be engineered to protect the results from being compromised by terrestrial microbial contaminants? Sun suggests using nutrients that promote only short-term respiratory activity without cell division. “The best scenario would be to find that the opposite enantiomers are consumed, enantiomers that terrestrial microbes would not touch,” he says.

Sun’s interest in homochirality and Mars grew from his research on endolithic microorganisms that live in rocks—specifically, from his experiences in the McMurdo Dry Valleys in Antarctica. The focus was on chirality of amino acids, and why microbial communities there contain elevated levels of D-amino acids. “If you hydrolyze an endolithic specimen in hydrochloric acid, you get not only L-amino acids, but also large quantities, sometimes up to 30–40%, of D-amino acids,” he says. “Our working theory is that these D-amino acids are . . . [from] remnants of bacterial cell-wall peptidoglycans, which accumulate in the endolithic system while the easily degradable proteins are recycled . . . The chiral signature of life is much more subtle than generally thought.”

Meanwhile, Gilbert V. Levin, who oversaw the Viking Labeled Release experiment, has been waiting more than three decades for the National Aeronautic and Space Agency (NASA) to send follow-up experiments to Mars, he says. In the interim, he worked as part of a U.S.-Russian team that designed the Mars oxidant (MOx) experiment, which included chemically coated fiber optic strands intended to detect oxidants, whose activity might account for the results from the 1976 labeled-release experiments.

Levin recommended including D- and L-cysteine films on two of the MOx fiber optics strands. It was an alternative chirality experiment to the one Sun recommends, but also is capable of revealing biological activity. “I had to disguise its real biological intent, because NASA Headquarters had repeatedly proclaimed that no life detection experiment would be funded, and we (the MOx team) had already been funded by NASA.” Now he recommends including L- and D-lactate and L- and D-alanine components as part of the next round of life-seeking experiments in space, and Sun goes along with taking this more comprehensive approach.

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

Can Harnessed Microbes Meet “Sniff Test” To Compete with Fossil Fuels?

With concerns about global warming and rising oil prices, there is renewed impetus behind efforts to harness microorganisms as a way of reducing worldwide reliance on fossil fuels. Some companies are keen on exploiting photosynthetic microorganisms, whereas others are counting on other ways to marshal microbial metabolic prowess.

Here are highlights describing several recent developments in which microbial activity is at the heart of the technology. However, critics continue to question whether these or other microbial-based technologies can meet critical “sniff tests,” such as scale-up and efficiency, to make them competitive with fossil fuels.

“Helioculture” depends on genetically engineered photosynthetic microorganisms to convert “widely available”—but undisclosed—chemical nutrients along with brackish or seawater into fuels and “solar chemicals,” according to Joule Biotechnologies of Cambridge, Mass. The raw material is not biomass, according to Joule’s CEO Bill Sims. “To a certain extent, you can consider our feedstock to be carbon dioxide,” he says. Solar energy is captured in a special “solar converter, [which] is a flat panel device and inside is a solution of non-fresh water, the nutrients, and highly engineered photosynthetic microorganisms.” Fermentation products include ethanol and hydrocarbons, some of which could be used for making plastics.

The Joule research team now plans to scale up its experiments for evaluation

Virus Implicated for Some Prostate Cancers

A virus that causes leukemia and sarcomas in mice is also present in malignant human prostate cancer cells, according to Ila Singha of the University of Utah in Salt Lake City, Robert Schlaberg of Columbia University Medical Center in New York, N.Y., and their collaborators. Specifically, the xenotropic murine leukemia virus (XMRV), which is a retrovirus, turned up “in 27% of prostate cancers we examined and was associated with more aggressive tumors,” Singha says. “We still don’t know that this virus causes cancer in people, but that is an important question we’re going to investigate.” The researchers also detected XMRV proteins in malignant prostate cells, further suggesting that XMRV infection is linked to tumor formation. If so, these findings could lead to new diagnostic tests, vaccines, and therapies for treating this cancer, which is diagnosed in nearly 200,000 men in the United States each year. Details appeared on 7 September online in the Proceedings of the National Academy of Sciences.
outdoors “somewhere in the South- west,” according to Sims. One near-term goal is to determine whether its helioculture approach can yield 20,000 gallons of ethanol per acre per year. If all goes well, plans call for locating a pilot plant in the same region because sunlight is abundant but the land is not well suited for agriculture.

Meanwhile, PetroAlgae in Melbourne, Fla., also is depending on sun-light to generate fuels via microorgan- isms such as algae, diatoms, and cyanobacteria that will be grown in “large, open-pond bioreactors,” says company representative Andrew Beck. “Our technology precisely man- ages exposure to light to dramatically increase the growth and productivity of indigenous microorganisms,” or “micro-crops.” They yield diesel fuel and also a high-protein liquid and a mash, rich in carbohydrates and lip- ids, that could be used as animal feed.

Last April, PetroAlgae signed a com- mercial licensing deal with GTB Power, a consortium in mainland China and Taiwan, while others are being negoti- ated elsewhere in Asia as well as in Eu- rope, the Middle East, Africa, and South and North America, according to Beck. The April agreement calls for building ten 5,000-hectare, commercial units in China starting late this year.

Sunlight does not appear to be part of the recent $10-million agreement between oil giant British Petroleum (BP) and Martek Biosciences of Co- lumbia, Md. The latter company is focused on microorganisms that can convert sugars into lipids through a heterotrophic process that requires neither direct sunlight nor carbon di- oxide. The sugars come from many sources, including feedstocks that BP is already using or investigating such as sugar cane, woodchips, or grasses.

One promising feature of the part- nership is that, for more than two decades, Martek has used its fermenta- tion technology on an industrial scale to produce lipids for infant for- mula and food supplements, accord- ing to company spokeswoman Cassie France-Kelly. “The BP/Martek ap- proach is reliant on technologies proven at scale, rather than technologies being explored by other companies in the lab stage of development,” she says.

However, some experts are skepti- cal about relying on microbially fer- mented sugars as a means for solving current dependence on fossil fuels to meet global energy needs. “The large- scale production of transportation fu- els will not be economical or sustain- able using sugar produced by photosynthesis as feedstock for micro- bial conversion to biodiesel,” says chemist Gerard C. Dismukes at Prince- ton University in Princeton, N. J., who also says that turning microalgal lipids into biofuels is not practical. “A direct process must be found.”

“Making a smooth transition to a world supported by sustainable re- sources is the defining challenge of our time, with transportation among the most important pieces of this chal- lenge,” says Lee Lynd, an environ- mental engineer at Dartmouth College in Hanover, N.H. “In looking for technologies that can achieve mean- ingful petroleum displacement, we need to apply critical sniff tests to make sure that there is real potential to achieve this aim.”

Barry E. DiGregorio

Coinfecting Viruses and Mycoplasmas Can Appear To Cooperate

Very different kinds of pathogen—in this case, one a virus, the other a myco- plasma—can act as if cooperating when infecting cultured cells, with one

In Microbiological Terms: No Guts, No Glory

Long underappreciated as a microbial habitat, the gastrointestinal (GI) tract is basking in the scientific limelight these days. Here are some recent examples:

• Engineering *Escherichia coli* bacteria to make GLP-1, a protein that stimulates cells in the pancreas to produce insulin, and then introducing the modified bacteria into the GI tracts of diabetic mice provides a way of treating that disease, according to biochemical engineer John March at Cornell University in Ithaca, N.Y., and his collaborators, who presented their findings during the August meeting of the American Chemical Society.

• In analyzing genes from the GI microflora of two healthy individuals, data based on DNA fragments encoding resistance genes “suggest that we have just begun to scratch the surface of the immense diversity of antibiotic resistance machinery in the human micro- biome,” report George Church of Harvard Medical School in Boston, Mass. and his collaborators, whose findings appear in the 28 August *Science*.

• Deer carrying the prion associated with chronic wasting disease excrete infectious prions in their feces long before they develop any symptoms of that disease, a finding that helps to explain how this disease spreads among deer, according to Stanley Prusiner of the University of California, San Francisco, and his collaborators, whose findings appear in the 10 September *Nature*.
augmenting the potency of the other, according to Peter Lidsky and Vadim I. Agol of the Russian Academy of Medical Sciences, Moscow, Russia, and their collaborators at the Academy and the nearby M. V. Lomonosov Moscow State University, as well as at Radboud University Nijmegen Medical Centre in Nijmegen, the Netherlands. Thus a DNAase from a simultaneously infecting mycoplasma enhances the cell-killing activity of the encephalomyocarditis (EMC) virus, they report in the October Journal of Virology (83:9940–9951).

The Russian and Dutch microbiologists were analyzing what happens when EMC virus infects cultured cells. They were following up earlier studies in which Agol and his collaborators learned that poliovirus sometimes activates—but at other times suppresses—apoptosis (cell killing) in cells that it infects. Their 1995 report not only describes this apoptosis-triggering activity, but also presents the first case of apoptosis suppression via an RNA virus with a simple genome, he says. Both poliovirus and EMC are picornaviruses. While poliovirus infects the gastrointestinal tract, EMC infects the heart, making it a cardiovirus and thus a member of a more recently recognized group of human pathogens, according to Agol.

What the Russian-Dutch team studying EMC did not realize at first is that some of their cell cultures were contaminated with Mycoplasma hyorhinis. Thus, sometimes EMC degraded host-cell DNA, in a manner similar to what happens during apoptosis. However, compounds that ordinarily suppress apoptosis failed to do so. The reason is that a DNAase from the mycoplasma was responsible for degrading the cellular DNA and, thereby, enhancing the potency of the viral infection. Lidsky, Agol, and their collaborators call this phenomenon involving such different pathogens “cooperation.”

Cardioviruses in mycoplasma-free cultures also trigger apoptosis, according to Agol. However, he says, the viral leader protein can suppress it, preventing DNA degradation in these cells.

“We initially observed that cardioviruses triggered in infected cells a very strong DNA degradation, which is a hallmark of apoptosis,” Agol says. “One day, Peter Lidsky came to me and said he had obtained evidence that this DNAase likely originated from mycoplasma, a frequent, undesirable contaminant of cell cultures. He and [another colleague] Lyudmila Romanova were extremely disappointed, thinking that their results should be thrown away.” However, they came to view the findings differently—as reflecting what likely occurs often in nature, simultaneous infection with viral and microbial agents.

Uncovering evidence for cooperation between such different pathogens is a “pioneering piece of work,” says Andrei V. Gudkov of Roswell Park Cancer Institute in Buffalo, N.Y., referring to the research of the Dutch and Russian microbiologists. “Their data are consistent with the hypothesis that the EMC virus infection ‘opens up’ the mammalian cell to DNAase of mycoplasma origin, thereby changing the cytotoxicity pattern of an otherwise quite benign mycoplasma infection.” He also wonders whether and how mycoplasma infections affect the titer of viral progeny. On the other hand, he adds, these findings may simply “represent a completely artificial clash between two parasites which provides no benefit for either.”

“However,” Gudkov continues, “the more interesting hypothesis is that this outcome of viral infection reflects an adaptive survival mechanism of mycoplasma. In fact, mycoplasma seems to induce an apoptosis-like outcome of viral infection, something that practically all viruses try to avoid.” The findings support the importance of maintaining healthy microflora, and the need for more conservative use of antimicrobials, he says. “I would not be surprised that one day we will have to conclude that subjects with chronic mycoplasma infections are more resistant to certain viruses.”