Host Factors Appear To Affect Impact of Therapeutic Vaccines

Administering a “therapeutic” vaccine only modestly enhances T cell responses to control lymphocytic choriomeningitis virus (LCMV) in mice chronically infected with this virus, according to E. John Wherry, formerly of Emory University School of Medicine in Atlanta, Ga., and now at the Wistar Institute, Philadelphia, Penn. “This was in striking contrast to the strong response to the vaccine... in healthy... mice,” he says, referring to findings that he and his collaborators published in the July issue of the Journal of Virology.

The main reason for the feeble response appears to be that host T cells, which the vaccine was designed to boost, fail to proliferate vigorously and respond only weakly, Wherry says. “T cells can become dysfunctional during chronic infections, losing antiviral functions. Importantly, mice with lower viral loads at the time of vaccination responded better, suggesting that the more severe the state of chronic infection, the more dysfunctional the responding T cells and the more poorly they will respond to therapeutic vaccination.”

“Therapeutic vaccination, in principle an appealing approach to enhance immune control in infectious and neoplastic disease, has been troubled by only minimal to moderate... benefits in a wide variety of experimental systems,” says Dirk Homann of the University of Colorado at Denver. Even so, Wherry’s studies “validate” an approach in which “the antigenic burden”—in this case, viral load—is lowered before administering the therapeutic vaccine to infected hosts, Homann points out. Thus, this combined approach in which other steps are taken to counteract the virus and boost the host immune response could “improve the quantity and quality of specific T cells induced by this therapeutic regimen,” he says.

“One observation in particular caught my attention,” Homann continues. “While the number of virus-specific T cells induced by the vaccine was variable and at best moderately increased, the T cells responsive to the vaccine appeared to demonstrate improved functionality. This... may result from the preferential expansion of a ‘more functional’ T cell subset.”

“Our future studies will focus on understanding the molecular mechanisms of T cell dysfunction that underlie poor responses to therapeutic vaccination,” Wherry says. “In addition, we will examine approaches where a therapeutic vaccine is combined with other therapeutics, such as cytokines or costimulatory molecules, that may be able to overcome the weak proliferative potential of the responding T cells.”

David Holzman
David Holzman writes from Lexington, Mass.

Several Recent Policy and Regulatory Developments Involve Microbiology

Several recent regulatory or policy developments pertain to microbiology, including:

- A U.S. Appeals Court in July overruled a federal Court in Montana to permit imports of Canadian beef cattle (ASM News, May 2005, p. 218). The U.S. border had been closed for such imports since May 2003 after a cow in Alberta tested positive for bovine spongiform encephalopathy.

- Officials from federal agencies, including the Centers for Disease Control and Prevention (CDC), Food and Drug Administration, and National Institutes of Health, said in July that vaccines for children that are preserved with mercury-containing thimerosal do not pose a risk of causing autism. However, Representative Dave Weldon (R-Fla.), who has urged federal officials to have that preservative removed from vaccines, continues to express skepticism over its safety.

- Officials from the U.S. Department of Agriculture in July issued the first license for a DNA-based vaccine—specifically, for an injectable product to protect horses against West Nile virus that was developed jointly by Fort Dodge Animal Health of Overland Park, Kans., and collaborators at CDC facilities in Fort Collins, Colo.

- The National Academies issued a report, “Animal Health at the Crossroads: Preventing, Detecting, and Diagnosing Animal Diseases,” calling for a new “high-level mechanism to coordinate the currently fragmented framework for confronting new and emerging animal-borne diseases” and also for “stronger links in the network of public and private labs that test for and diagnose animal diseases.”
(NIAID), a component of the National Institutes of Health (NIH), in partnership with ASM, conducted a survey of academic, biotechnology, and pharmaceutical—but not federal—entities in the United States regarding the location, capacity, and status of domestic laboratories with biosafety level 3 (BSL-3) containment facilities and equipment. BSL-3 containment is used in clinical, diagnostic, teaching, research, and production facilities in which work is done with microbial agents that may cause serious or lethal disease.

Representatives from NIAID and ASM developed their approach for conducting this survey, and subsequently received approval from the federal Office of Management and Budget (OMB) to proceed. ASM then arranged with Constella Health Sciences of Atlanta, Ga., for technical support in conducting the survey and analyzing its data. Because the purpose of the survey was to provide NIAID with information for planning purposes, there are no plans to maintain or update this database.

Survey solicitations were mailed to 1,096 environmental health and safety officers (or their equivalents) at academic, biotechnology, and pharmaceutical facilities. Respondents were asked questions that addressed the number and size of the BSL-3 capable laboratories, the current type(s) of work being conducted within the laboratories, and their research capabilities. Responses received by 9 March 2005 were included in the survey database. A secure online submission form was developed to protect the integrity of the responses and respondents. In addition, all survey recipients were informed that their submitted responses are exempt from disclosure under Exemption 4 of the Freedom of Information Act (FOIA), as provided in the Department of Health and Human Services (DHHS) regulations implementing the FOIA.

To be sure of the comprehensive-ness of the survey, OMB called for a validation study to determine whether the respondents accurately represent U.S. facilities. This validation study entailed randomly sampling ASM members to determine if there are laboratories at their institutions that meet BSL-3 criteria set forth in the survey. Because 100 facilities received both survey and validation study requests, the population identified for the survey appears to be an accurate reflection of the universe of facilities in the United States likely to have BSL-3 laboratory capabilities.

Of the 1,096 facilities that were mailed surveys, 528 (48.1%) responded. Among these respondents, 52.3% represented academic institutions, 6.9% were industry/commercial, 17.3% were clinical/diagnostic, and 28.9% were classified as “other.” Nearly half (245, or 46.4%) of the responding facilities indicated that they currently have BSL-3 capable laboratories, while the remaining 283 indicated that they do not.

In total, 598 individual laboratories are associated with the 245 facilities that report BSL-3 capabilities. Responses indicated that there are nine or more facilities having BSL-3 capability in eight states (California, Washington, Wisconsin, Pennsylvania, New York, Connecticut, Tennessee, and Florida), while another three states (Texas, Maryland, and Indiana) have between seven or eight facilities with that capability. Most facilities with BSL-3 capability have three or fewer laboratories, although one institution reported having 30 such labs.

Nearly 45% of facilities with BSL-3 capacity reported that they were capable of handling small-animal studies, but only 2.9% are capable of conducting nonhuman primate studies at the BSL-3 level. Good Laboratory Practice capability was reported by 23.3% of the facilities with BSL-3 laborato-
Change that Metaphor: Microbes Belong Not to a Family Tree, but to a Net

Quantitative analyses of evolutionary relationships suggest that a sensible way to present relationships among microorganisms is as part of a net instead of the traditional metaphor of a tree, according to Christos Ouzounis, leader of the Computational Genomics Group at the European Bioinformatics Institute in Cambridge, England, and his collaborators. Their report, published in the June issue of Genome Research, attempts to account for extensive horizontal gene transfers among microorganisms and then presents them as interconnecting vines in a complex network with two main microbial branches—one for Bacteria and the other for Archaea. Within this tabulation of more than 600,000 vertical transfers, 90,000 gene losses, and about 40,000 horizontal gene transfers, a few species, particularly the nitrogen-fixing soil bacteria, “appear to be champions of horizontal gene transfer,” he says, noting: “It’s entirely possible that apparently harmless organisms are quietly spreading antibiotic resistance under our feet.”

Shocking: Both Gram-Positive and -Negative Bacteria Generate Electricity

Gram-positive, spore-forming Desulfitobacterium hafniense strain DCB2 can generate electricity in experimental fuel cells, according to Charlie Milliken and Harold May of the Medical University of South Carolina, Charleston, who presented their findings at the poster session “General Environmental Microbiology—Part 1,” during the 105th ASM General Meeting in Atlanta, Ga., last June. Equally remarkable, gram-negative Geobacter species apparently use external pili to transmit electrons to electrode surfaces in comparable fuel cells, according to Derek Lovley of the University of Massachusetts (UM), Amherst, who spoke during the symposium, “Revelations from Bacterial Genomes.”

“We were looking at the degradation of halogenated hydrocarbon compounds, and were trying these gram-positive bacteria,” May says. “We also knew that they could reduce metals.” Out of curiosity, he and Milliken introduced D. hafniense cells into a miniature fuel cell, and quickly determined that they could produce a low-level electric current. The power output is modest, in the range of 2–10 mW/m² of anode surface. Noting that the current setup is “fine for testing microorganisms,” the two scientists say that they are collaborating with electrochemists at Clemson University to redesign the fuel cells with improved materials for greater electrical production efficiencies.

The bacteria appear to form bio-films along the electrode surfaces and remain viable during intermittent periods when nutrients are exhausted and then replenished. “We’re not sure it could last for a whole year, but it lasts for days,” Milliken says. Moreover, because these bacteria can form spores, they have another potential means for reactivating cells over more extended periods.

“We don’t know exactly how they do this, but the living cells are in contact with the electrode, and they need ‘fuel’ in the form of growth media, and some work, while others don’t,” May says. “The DCB2 cells go to the anode in an anaerobic zone, and if we substitute substrates [in the growth medium] that they don’t like to use, they stop producing current.”

The bacteria can be a bit finicky in this regard, Milliken adds. For instance, although such cells will grow using butyrate as a carbon source, they will not produce electricity when this fatty acid is provided within the experimental fuel cell. However, although cells cannot grow when supplied with hydrogen gas, they continue to generate electricity in the presence of this gas, May adds.

In other ways, the bacteria are far from finicky, according to May. “We get buckets more power from the fuel cells if they don’t contain a ‘pure’ but instead contain an ‘enriched’ culture, even though we don’t know for sure what else is in there, except a bunch of gram-positive rods,” he says, noting that other researchers studying fuel cells similarly report that mixed, rather than pure, microbial cultures tend to be more efficient in producing electricity.

Meanwhile, Lovley and his UM colleagues are trying to determine how electrons inside gram-negative Geobacter cells move across the inner and outer membranes to electrodes in fuel cells. Much of the early focus was on cytochrome proteins because such cells encode more than 100 cytochrome genes, he says. Although sev-
eral cytochrome proteins, with at least one of them acting as an electron shuttle, appear to be moving electrons from the inner to the outer membrane, they do not appear to be involved in transferring electrons from the outer membrane to external electrodes.

Frustrated with cytochromes, Lovley and his colleagues considered the appendages of Geobacter. In particular, “extremely fine” pili that are a mere 3–5 nm in diameter but extend 20 μm beyond the outer membrane of such cells seem to be responsible for reaching electrode surfaces and delivering electrons. “We joked that maybe these pili are like wires,” he says. But various measurements indicate these Geobacter proteins are indeed “highly conductive” and very much like wires, whereas those from other bacteria, such as Pseudomonas aeruginosa, are not. “We don’t know how they conduct, we just know they do,” he says. “Such long, thin, conductive structures are unprecedented in biology,” Lovley says. “This completely changes our concept of how microorganisms can handle electrons, and it also seems likely that microbial nanowires could be useful materials for the development of extremely small electronic devices.”

Jeffrey L. Fox

Electron Tomography Details Bacterial Structures

Electron microscope (EM) tomography helps to bridge the gap between X-ray and nuclear magnetic resonance, and provides molecular-level details of key structures in bacterial cells, says Sriram Subramaniam of the National Institutes of Health (NIH) in Bethesda, Md. He and Martin Kessel, also of NIH, co-organized the symposium “Electron Tomography—an Exciting New Way to Explore the 3-Dimensional Structure of Microbial Cells,” convened during the 105th ASM General Meeting in Atlanta, Ga., last June.

EM tomography now offers unparalleled images of critical phases during the complex life cycle of Caulobacter crescentus cells, says symposium participant Ken Downing of the Lawrence Berkeley National Laboratory (LBNL) in California. For instance, using tomography, he and his collaborators identified a “tunnel” that forms transiently in both the outer and inner membranes “shortly before each membrane separates to the daughter cells.” The tunnel is particularly well defined between the inner

Bits and Pieces about Pathogens and Parasites of Plants and People

When it comes to pathogens and parasites that affect people and plants, there is plenty of pertinent progress, including:

• The mitogen-activated protein kinase of the rice blast fungus, Magnaporthe grisea, “controls penetration. . . like driving nails through the plant surface” and thereby “beginning a signal transduction pathway” that eventually can reduce crop yields by 75%, according to Jin-Rong Xu and his collaborators at Purdue University in West Lafayette, Indiana, who report their findings in the May issue of Plant Cell.

• When tested in mice, the anti-cancer drug Gleevec inhibits otherwise lethal doses of vaccinia virus, a relative of smallpox virus, by blocking access to cell receptors from the family of Abl proteins, according to Daniel Kalman of Emory University School of Medicine in Atlanta, Georgia, and his collaborators who report their findings in the July issue of Nature Medicine.

• Both the Nipah and Hendra viruses use the receptor called Ephrin-B2 to bind to and enter cells of the central nervous system and those lining blood vessels, according to Benhur Lee of the University of California, Los Angeles and Christopher Broder of the Uniformed Services University of the Health Sciences in Bethesda, Md., and their respective colleagues, who report their findings, respectively, in Nature and Proceedings of the National Academy of Sciences during the second week of July.

• Hemoglobin C disrupts how PfEMP-1, a protein of malaria parasites, is distributed along surfaces of infected red blood cells, reducing their stickiness and apparently explaining why humans carrying this version of hemoglobin are better able to withstand this disease, according to David Wellems of the National Institute of Allergy and Infectious Diseases in Bethesda, Md., and his collaborators, who report their findings in the 23 June issue of Nature.

• Researchers from more than 20 laboratories reported determining the genomic sequences for the parasites, Trypanosoma brucei, Trypanosoma cruzi, and Leishmania major, that respectively cause three insect-borne diseases—African sleeping sickness, Chagas disease, and leishmaniasis. Despite major differences, these three parasites have a common core of 6,200 genes, according to the reports that appear in the 15 July issue of Science.
membranes, according to Downing. Whether the tunnel is a transient and functionless consequence of cell separation or truly a usable (and used) conduit is up for study.

EM tomography also proves useful for studying Spiroplasma, the smallest known free-living and self-replicating cells, that otherwise would be difficult to visualize because they “are at the limiting [range] of the resolving power of the light microscope,” says Shlomo Trachtenberg of Hebrew University in Jerusalem, Israel, who focuses on the “acrobatic” swimming of such cells. EM tomography reveals that these spiral-shaped bacteria depend on a lengthwise ribbon of conformationally variable fibrils composed of subunit tetramers to flex, deform, and extend-contract, or “breathe,” he says. “Three-dimensional tomography has been essential in revealing the presence of the ribbon.”

Before being subjected to EM tomography, specimens are flash frozen and sliced into thin sections that are tilted at different angles in the electron beam to generate multiple views. Those images are stored and combined electronically to generate three-dimensional images that “depict molecular orchestrations that underlie function,” according to symposium participant Stephan Nickell of the Max-Plank-Institute of Biochemistry in Martinsreid, Germany. “Molecular topologies are unique; no two cells are identical to each other,” he says. “Electron tomography allows the level of analysis necessary to permit statistical analysis of cellular organization...because it provides molecular resolution inside a cell.”

Bruce McEwen of the New York State Department of Health in Albany has used EM tomography to reveal the presence of fibril ribbons in the cytoplasm of Treponema pallidum, the corkscrew-shaped bacteria that cause syphilis. Although he detects no fibril-fibril bridges, the filaments appear to contain “bridging structures” that connect the ribbons to the cytoplasm. Although he speculates that the ribbons have a cytoskeleton function, he also cautions that “the exact relevance of the images is still being assessed, since frozen hydration can introduce a lot of noise.”

The flash-freezing step, which can limit the resolving power of EM tomography, may yield to another means of thin sectioning being explored by Subramaniam and his collaborators. Using a dual-beam electron microscope that
Members of the National Science Advisory Board for Biosecurity (NSABB), who met for the first time in June near Washington, are charged with guarding biological research activities against abuses by bioterrorists. But NSABB members also are seeking to continue encouraging researchers to pursue research, publish findings, and develop products. Dealing with this delicate “dual-use” issue promises to be a search for the right “balance,” board members and other experts readily agree.

Based on recommendations from a National Academy of Sciences (NAS) panel, NSABB was designed with the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC) in mind. Central to this strategy for dealing with biosecurity-related issues is development of a comparable advisory, quasi-regulatory scheme, with NSABB at its hub. At the June meeting, NSABB members formed several working groups to pursue several issues, including defining more precisely dual-use research, developing codes of conduct for researchers to follow, exploring communication (including publication) and international issues, and scrutinizing research efforts aimed at fabricating genes and genomes.

However, beyond forming working groups, it is not obvious how NSABB will deal with the broad array of biosecurity issues it faces, particularly because they need to be tackled on an international basis, says microbiologist Ronald Atlas, a graduate dean at the University of Louisville, Kentucky. “It’s important for researchers to develop a sense of ethics, and for this to come from the ground up, not from the administration and Congress down,” he says. What is being sought is not so much laws and rules as a “culture of responsibility,” agrees Gerald Epstein, a senior fellow with the Center for Strategic & International Studies in Washington.“Some deride that idea, but I think it’s the only way to go. If we try to jam this down peoples’ throats, it will fail.”

Another component of this balancing act is to “do no harm” to science, which is “fragile and easy to damage,” says board member David Relman of Stanford University in Stanford, Calif. “We publish thousands of papers per year with the potential for problems,” adds Lynn Enquist of Princeton University in Princeton, N.J., and editor in chief of the ASM Journal of Virology. “But there is a disconnect between information in papers and potential misuse...and we don’t want to stifle the scientific enterprise.” ASM editors review and flag manuscripts that raise biosecurity warning “flags,” he points out. Although a very few manuscripts were rewritten because of biosecurity concerns, none has yet been rejected or withheld on that basis.

Yet several board members, including Arturo Casadevall of Albert Einstein College of Medicine in Bronx, N.Y., and Paul Keim of Northern Arizona University in Flagstaff, say that research in microbiology already is being damaged. Both the select agent rule and intensified background checks on researchers who are foreign nationals “slow progress” even though they are not intended to impede research, Keim says. “A lot of work is seriously inhibited and cannot get done,” adds Casadevall.

Researchers working in industry share these concerns, says John Mulligan, president and CEO of Blue Heron Biotechnology, Inc. in Bothell, Wash., that does custom syntheses of genes. He says that current “select agent” rules “need improvement” because they aim at particular microbial species instead of specific gene sequences. “Nefarious uses [of synthetic genes] are certainly possible, but direct isolation of pathogens is an easier way to obtain them,” he says.

Overregulating could encumber legitimate clients who seek to develop vaccines, therapeutics, and other countermeasures, while merely driving illicit activity elsewhere, Mulligan says. And, adds Craig Venter, president of the J. Craig Venter Institute in Rockville, Md., where researchers attempt to assemble novel microbial genomes from fabricated components, “Meaningful research on pathogens is critical...[and] if we’re not concentrating our efforts on...countermeasures, we’re missing the big picture.”