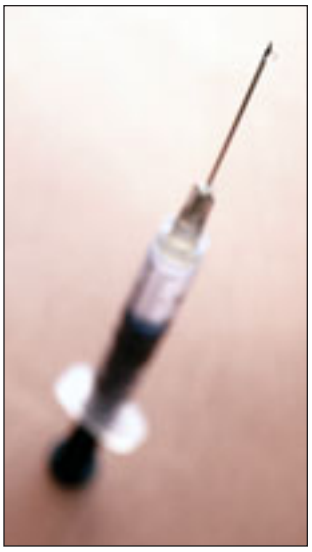

PHOTODISC

HIV vaccine testing

Although it's been 20 years since the HIV virus was identified and numerous therapies have emerged, no HIV vaccine yet exists. However, clinical trials initiated through collaborations between government and industry are moving ahead.

The Walter Reed Army Institute of Research (WRAIR) initiated the latest HIV vaccine clinical trial in May. The Phase I trial is evaluating the safety and immunogenicity of LFn-p24 vaccine, developed by WRAIR and AVANT Immunotherapeutics, at three escalating dose levels in 18 healthy adult volunteers.

LFn-p24 is based on AVANT's Therapore technology, which uses bacterial vectors, removed of their toxins, to deliver target antigens into human cells and induce a cell-mediated immune response.

"The company believes this technology offers significant advantages over other immunotherapeutic methods," says Una Ryan, president and CEO of AVANT.

Specifically, the vaccine consists of a detoxified anthrax-derived polypeptide, called lethal factor, fused to the HIV-1 gag P24 protein. In vivo tests of this vector, according to AVANT, have demonstrated cellular delivery of peptides that stimulate potent immune responses.

Merck also has an HIV vaccine using a biovector in early development. In September 2003, the company began an international Phase I trial for its vaccine that uses replication-defective adenovirus (which causes the common cold) to express the HIV *gag* gene.

Yet another approach is based on DNA vaccines, which recently entered trials in the form of Chiron's microparticle-delivered *gag* DNA plasmids. The biotech firm began U.S. trials in January for the DNA vaccine in combination with a protein-based formulation.

Each of these trials is the result of collaboration between industry and the U.S. government. WRAIR and the NIH are involved in the AVANT study, while the HIV Vaccine Trials Network (HVTN), formed by the NIH in 1999, is working on the Merck and Chiron projects.

"The global AIDS epidemic will continue to require strong public–private partnerships to address this growing threat to world health," Chiron president John Lambert says.

The HVTN reports 11 vaccine trials in progress and about 15 planned to begin in the near future.

However, the most advanced vaccine candidate, AIDSVAX, a recombinant HIV gp120 protein developed by VaxGen, failed to improve HIV protection in clinical trials performed in North America, Europe, and Thailand, according to results announced in 2003.

—DAVID FILMORE

Leave a message

In seeking to isolate and characterize a human tRNA maturation complex, PTC Therapeutics senior scientist Christopher Trotta and colleagues may have stumbled across a unified RNA splicing mechanism that can serve as a drug discovery target (*Cell* **2004,** *117,* 1–20).

Most eukaryotic mRNA molecules go through a maturation process, in which endonuclease complexes excise short nucleotide sequences called introns. This is also true, to a lesser extent, of tRNA molecules, which are involved in translating the mRNA message into protein sequences. Although proteins involved in the tRNA maturation complexes of many eukaryotes are well characterized, human complexes are not.

Initially, the researchers used a BLAST search to look for human versions of the four yeast proteins involved in tRNA maturation, finding homologues for three of the four. Interestingly, when they generated cDNAs of the three sequences using PCR, they found that one (SEN2) occurred in two forms, with one shorter than expected (SEN2ΔEx8). They then used these sequences to generate tagged copies of two of the subunits and isolated active complex from human tissues using affinity chromatography. They found that while complexes containing SEN2 generated predicted RNA splice sites, those containing SEN2ΔEx8 produced altered splice sites.

The researchers used SDS-PAGE analysis to look for other proteins involved in the human tRNA maturation complex. Using MS and amino acid sequencing, they identified an 18-kDa protein as the last human homologue of the yeast complex proteins. Surprisingly, they also noted Clp1 protein, which was originally isolated as a component of an mRNA splicing complex, suggesting that the two complexes might share activities. Using antisense RNA to deplete SEN2 levels by almost half, the researchers found that both tRNA and mRNA maturation was inhibited.

"Researchers have always treated these processes as entirely separate sets of reactions," says David Engelke, director of biomedical sciences at the University of Michigan. "It is now clear that they are intertwined."

"We are excited by the discovery of this novel enzyme complex," adds PTC Therapeutics president and CEO Stuart Peltz, "and its implications in the identification of therapeutics that target novel mechanisms of post-translational control processes."

—RANDALL C. WILLIS

Lithium and AD

Lithium inhibits production of a major component of senile plaques associated with Alzheimer's disease, according to recent findings by scientists at Lilly Research Laboratories and Indiana University Medical School.

Physicians have prescribed lithium to treat bipolar disorder for decades, but the therapy's underlying molecular mechanism has never been fully elucidated. Recent studies, however, suggest the drug might act by inhibiting glycogen synthase kinase-3β (GSK3β), which is involved in processing several neurologically important proteins.

Eli Lilly researcher Yuan Su and colleagues examined what effect lithium and another neuromodulatory drug, valproic acid, might have on the amyloid precursor protein (APP) and β amyloid (Aβ), important species in the onset of Alzheimer's disease (*Biochemistry* **2004,** *43,* 6899–6908).

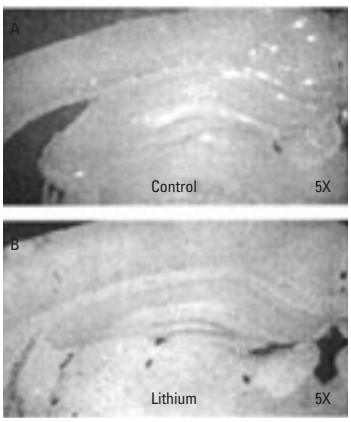
The team treated Alzheimer's mice models that overexpress human APP with lithium and valproic acid. Using Aβ-specific ELISAs on brain sections, they found that either drug significantly lowered Aβ levels at therapeutically relevant doses and in a dose-dependent manner. In vitro, they observed that APP levels are unaffected by drug treatment, but Aβ levels remain low after lithium or valproic acid administration. These findings suggest the drugs affect APP processing, and the researchers turned their attention to GSK3β.

They transfected HEK cells with either a dominant negative GSK3β construct or a specific antisense oligonucleotide to disrupt GSK3β function. They found that either treatment resulted in significantly lower Aβ levels, which mimicked the lithium and valproic acid results and suggested a possible role of GSK3β in APP processing. The researchers subsequently transfected mice with a construct that overexpresses GSK3β and found these mice showed a significant increase in Aβ levels. This effect could be ameliorated with lithium.

The scientists then examined whether dietary treatment of the mouse model for Alzheimer's could abrogate the onset of disease, which normally occurs at 3–4 months of age. They started feeding lithium carbonate to some of the mice at 1 month of age and continued treatment for 7 months. Lithium-fed mice showed significantly lower Aβ levels.

"Our findings," the authors wrote, "suggest that agents that directly or indirectly inhibit GSK3β might be effective as therapeutic interventions."

—RANDALL C. WILLIS



Braindrain: Compared with control (A), the brain section of a lithium-fed mouse model of Alzheimer's disease (B) shows significantly reduced amyloid plaque formation. (Adapted with permission from Su, Y.; et al. *Biochemistry* **2004,** *43,* 6899–6908.)

BioShield strikes back?

On the heels of the Senate passage of the Project BioShield Act in May (more than a year after the legislation was introduced), leading senators began pushing for new legislation seeking to make the term "defense contractor" just as appropriate for Pfizer and Merck as it is for Lockheed Martin and Boeing.

BioShield directs $5.6 billion over 10 years to purchasing vaccines, drugs, and other countermeasures against potential biological terrorism agents as an incentive for industry to develop such products. It also gives the NIH greater authority and flexibility in awarding biodefense R&D contracts and allows the FDA to temporarily release an experimental product for expanded use in an emergency.

Several companies, including VaxGen, Avecia, Acambis, and Bavarian Nordic, are already actively pursuing BioShield money aimed at stockpiling anthrax and smallpox vaccines.

LIEBERMAN PRESS OFFICE

Sen. Joseph Lieberman is looking to "create the right conditions" for a biodefense industry.

BioShield passed by wide margins in the House and the Senate. But many in Congress and industry don't think it goes far enough. Sens. Joseph Lieberman (D-CT) and Orrin Hatch (R-UT) are promoting another measure they and others are calling "BioShield II."

The sequel, which would be based on elements of the Biological, Chemical, and Radiological Weapons Countermeasures Research Act the two senators originally introduced in 2002, aims to "create the right conditions" to generate a biodefense industry, they say. This includes providing tax incentives, assuring intellectual-property rights, and increasing liability protections for companies producing countermeasures.

"We will know that we've established a biodefense industry when hundreds of millions of dollars in company and investor capital are available to fund countermeasure research, and investors see a reasonable opportunity to profit to the same degree they do on investments in other biomedical research," Lieberman and Hatch wrote in the May 19, 2004, issue of *The Hill*.

The pharmaceutical and biotechnology industries are hoping for more attractive incentives in the new bill. One idea that is being floated, says Frank Rapoport, a Pennsylvania-based attorney and industry lobbyist, is the "patent extender," in which a company would be able to pick a drug to receive an extended patent life if the firm invests a certain level of resources in biodefense projects.

"These are the kinds of bells and whistles that did not get into BioShield," Rapoport says. "BioShield is a very plain vanilla."

How much such bells and whistles will cost is not yet clear.

—DAVID FILMORE

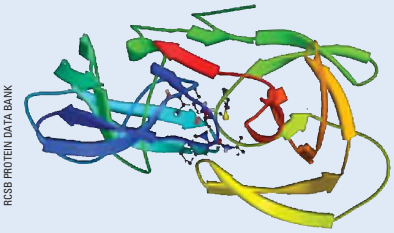
Structural alliances

Big Pharma is adding more structure to its activities. Roche, Eli Lilly, and others have recently formed or furthered partnerships with firms focused on structure-based drug design, in which leads are fashioned to three-dimensional protein shapes.

HIV protease inhibitors Viracept and Agenerase and the flu treatments Relenza and Tamiflu are drugs notable for being discovered via a structure-based process. But, by and large, discovery activities over the past decade have centered on diversity-based combinatorial chemistry and high-throughput screening. Fitting a drug directly to a target protein has not been feasible in many cases, because of the often long and challenging process of obtaining protein crystal structures.

However, several companies, including Syrrx, Structural GenomiX (SGX), and Astex Technology, are combining automation with advances in crystallography techniques to pursue high-throughput X-ray crystallography (HTC) in facilitating structure-based drug design.

In May, Syrrx signed onto an alliance with Roche to discover and develop cancer and Type 2 diabetes drug candidates. Syrrx seeks "to be the first organization to determine the three-dimensional structure of known drug targets." This agreement, which, Syrrx says, could reach a value of $178 million, centers on the oncology target HDAC and the metabolic target 11-β HSD-1.



RCSB PROTEIN DATA BANK

Nice fit. Viracept (nelfinavir mesylate), shown here complexed to its target HIV-1 protease, is a model for structure-based drug design.

Meanwhile, SGX has collaborated with Eli Lilly since April 2003 to provide structures of Lilly drug targets, generate data on compounds that bind to the targets, and install a high-throughput structural biology facility allowing Lilly to perform its own experiments. In January 2004, the partnership was expanded to give Lilly access to SGX's "third-generation" synchrotron beamline, an important tool for rapid protein crystallography.

In March, SGX provided its FAST technology to the biotechnology company Serono. The FAST process involves crystallographic screening of potential drug fragments and structure-guided elaboration of the fragments into full-fledged lead molecules.

Also in March, Boehringer Ingelheim entered an alliance with Astex to use its HTC and fragment-based discovery approach, called Pyramid, to generate leads for Boehringer's targets. Astex announced progress in April in a similar collaboration with AstraZeneca to target Alzheimer's disease. Astex is working with Schering AG and Aventis Pharmaceuticals on structure-based design projects as well.

Major challenges remain for producing crystal structures of membrane proteins, the majority of today's validated drug targets. But continued efforts in HTC are making 3D protein structures a more routine component of early-stage discovery programs.

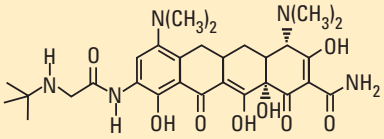
—DAVID FILMORE

Tigecycline trials

The first of a new class of antibiotics is showing promise as it moves through advanced clinical development.

Tigecycline—an injectable glycylcycline—a class of tetracycline derivatives that inhibit bacterial protein synthesis and cell growth—produced a 74% cure rate in hospitalized patients with so-called "complicated skin and skin structure infections" (cSSSIs) in Phase II study results announced in May. The drug, in development by Wyeth Pharmaceuticals, also caused a bacterial eradication rate of 70% at 50-mg dosage levels.

"Tigecycline displayed promising efficacy against a wide spectrum of bacteria with an acceptable safety profile," says study leader Russell Postier, professor of surgery at the University of Oklahoma.



Tigecycline is the first glycylcycline antibiotic to reach Phase III trials.

Hospitalized patients are particularly vulnerable to cSSSIs—such as infected ulcers, major abscesses, or superficial infections—and to infection-related death. These infections can be caused by various bacterial strains, including staphylococci, streptococci, and other less common organisms, both susceptible and resistant to currently approved antibiotics.

According to the U.S. Centers for Disease Control and Prevention, 70% of bacteria causing hospital-acquired infections are resistant to at least one common antibiotic. Wyeth is trying to establish tigecycline as a broad-spectrum treatment that can handle currently resistant strains.

The antibiotic has demonstrated effectiveness in laboratory in vitro tests against methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis,* and *E. coli,* among other troublesome organisms. Wyeth is conducting multicenter Phase III trials it says will be completed this year. In these studies, researchers are comparing the drug with other antibiotics for treating MRSA, vancomycin-resistant *Enterococcus*, and intra-abdominal infections in hospitalized patients.

Most recently, the company commissioned International Health Management Associates (IHMA) to conduct the Tigecycline Evaluation and Surveillance Trial (T.E.S.T), which will evaluate the antibiotic's activity against a wide variety of bacteria in vitro in nearly 700 hospitals in about 35 countries over three years.

"T.E.S.T. is likely one of the largest surveillance studies of its kind ever undertaken," says Daryl Hoban, IHMA director of clinical and laboratory services.

"This study will enable us to document the activity of this new antibiotic against bacterial pathogens, including those exhibiting multiple antibiotic resistance."

—DAVID FILMORE

Riding the nanotube

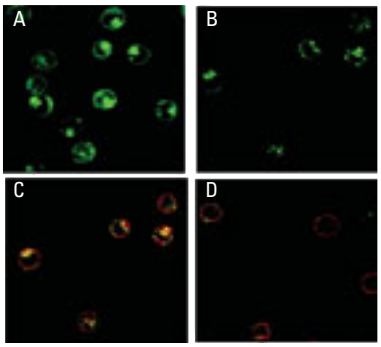
The promise of single-walled carbon nanotubes (SWNTs) as drug carriers got a boost with recent findings by Honglie Dai and colleagues at Stanford University. The researchers provided direct evidence of the uptake of nanotubes into human cells and identified a mechanism.

Drug delivery vehicles, such as lipid and polyethylene glycol derivatives, have been investigated to improve the cell penetration of many new drugs and biologics. Carbon nanotubes have also been discussed as potential delivery agents, but little data has been obtained about their ability to function as biocompatible drug transporters.

Dai's team prepared a homogeneous population of SWNTs, ranging from 100 to 1000 nm in length and 1 to 5 nm in diameter, that were extensively functionalized with carboxylate groups to allow for secondary molecule conjugation (*J. Am. Chem. Soc.* **2004,** *126,* ASAP).

The researchers fluorescently labeled the SWNTs and incubated them with human leukemia cells. Using confocal microscopy, the scientists noted significant fluorescent labeling both on the surface and in the cell cytoplasm. To test whether the nanotubes could transport macromolecules into cells, they labeled the nanotubes with fluorescently labeled streptavidin, a 60-kD protein, using a biotin linkage. Microscopy and flow cytometry measurements indicated rapid cellular uptake of the SWNT–biotin–streptavidin complex during incubation.

The scientists also used flow cytometry to determine whether nanotubes were toxic to various cell types, by looking for evidence of cell lysis. They found that SWNTs alone or singly conjugated did not precipitate cell death, even after prolonged exposure, but incubation of cells with the streptavidin conjugate exhibited toxic effects within 12 h. The researchers were able to alleviate this toxicity by simply lowering the streptavidin concentration.



Cell labeling. Using confocal microscopy, researchers examined the cellular uptake of fluorescently labeled nanotubes (green) and endosomes (red). (Adapted with permission from Kam, N. W. S.; et al. *J. Am. Chem. Soc.* **2004,** *126,* ASAP.)

Believing that endocytosis mediated cellular uptake, the scientists co-incubated cells with green-labeled SWNT conjugates and a red fluorescent marker that specifically stains endosomes. Using confocal microscopy, they observed yellow staining of the cells, providing direct evidence of nanotube endocytosis.

The researchers believe, "The biocompatibility and the unique physical, electrical, optical, and mechanical properties of SWNTs provide the basis for new classes of materials for drug, protein, and gene delivery applications."

—RANDALL C. WILLIS

Measuring immune-response memory

Researchers at the La Jolla Institute for Allergy and Immunology have discovered proteins that enable immune system T cells to retain memory of an infection. The findings, say the researchers, led by Hilde Cheroutre, could lead to immune-based therapeutics and vaccines.

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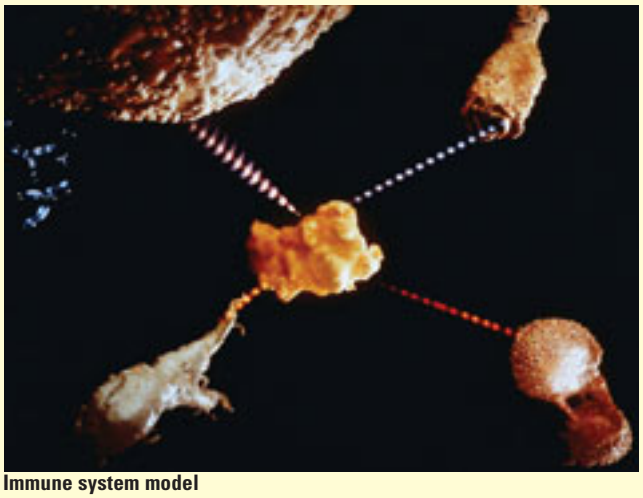

Immune system model

How memory T cells are activated during an immune response has been a long-standing question. T cells derive from bone marrow stem cells. Around the time of birth, lymphocytes leave the marrow and pass to the thymus gland, where they multiply and regulate immune system defenses.

Most T cells responding during a primary immune response subsequently undergo programmed cell death. However, a few activated cells survive as memory cells, which can persist for an individual's life and will later act rapidly against the same infection to provide immediate protection.

The La Jolla Institute researchers discovered two molecules, the thymus leukemia antigen and a subset of the CD8 protein, CD8αα, produced by some T cells during the immune response that provide survival signals to the cells. These proteins, the scientists say, can be used to identify T lymphocytes that will develop into long-lived memory cells.

Monitoring these markers might also provide a better approach for probing the effectiveness of vaccines or other therapies. The conventional measure for assessing immune memory has been the magnitude of the initial burst of T lymphocytes, the researchers point out.

"With more of these memory cells, the body can better fight the infection should it arise again," Cheroutre says. "This has major implications for the development of vaccines that will protect people longer and more effectively against disease."

—KIMBERLY S. CLEAVES ■