Medical College of Georgia

TOMORROW

2007

MCG’s mission is to improve health and reduce the burden of illness in society by discovering, disseminating and applying knowledge of human health and disease.
Combining bioinformatics and mouse models to better understand diabetes ... understanding how the immune system is programmed ... determining how neurons are connected....

These are among the many Medical College of Georgia research breakthroughs highlighted in this 2007 edition of MCG Tomorrow.

This annual magazine disseminates news of MCG’s important laboratory findings, particularly in our thematic areas of cancer, cardiovascular disease, diabetes/obesity, infection/inflammation and neurological disease.

Even amid an increasingly competitive research funding environment, MCG continues to make great strides, with all indicators pointing up. The past fiscal year marked a 3.9 percent increase in sponsored research funding for a total of $64 million. The university’s National Institutes of Health funding increased 4.6 percent to $45.2 million and its total number of sponsored research awards increased 25.3 percent. Thirty-six MCG scientists submitted invention disclosures or were named on patents, and research findings were publicized in dozens of prestigious scientific journals.

I take great pride in using this magazine to share the many implications behind these statistics: the lives that are improved, the years extended and the suffering alleviated through MCG research.

Rest assured that MCG research findings will benefit people for generations to come—and that the breakthroughs will just keep coming.

Daniel W. Rahn, M.D.
President, Medical College of Georgia
Senior Vice Chancellor for Health and Medical Programs, University System of Georgia

Dear Readers,
Check and Balances
Genes provide insight into schizophrenia and seizures.

Special Delivery
A motor protein is vital in connecting neurons.

Recovery in Motion
Stem cell transplants improve stroke outcomes.

In Control
Transplanted brain cells hold promise for Parkinson’s disease.

A Helping of Hemoglobin
A genetic variation may reduce the risk of Alzheimer’s disease.

Energy Booster
Creatine may be a shot in the arm for dying cells.
An Investment in the Future

As the articles in MCG Tomorrow make clear, biomedical research is all about the future: investments today that improve health tomorrow.

Most of those investments—inspiration, hard work and dedication, to name a few—are intangible. But tangible investments are also vital in helping MCG perpetuate its mission of improving health and reducing the burden of illness in society.

We ask that you consider partnering with us in this endeavor. Many methods are available to maximize your contributions and minimize your financial burden, including naming opportunities for MCG facilities, research endowments that can be directed to your area of interest, gifts in memory of a loved one and trusts that pay income to a designated individual for life.

MCG acknowledges gifts through several giving clubs, including the President’s Club, Milton Antony Guild and Society of 1828, which offer unique opportunities to plan your giving. Don’t hesitate to contact me for more information or to begin investing in MCG’s future today.

Tony Duva
Associate Vice President for Gift Planning
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Dear Readers,

Approximately two years ago, I began my tenure as vice president for research at the Medical College of Georgia.

What an adventure it has been, full of advances that only a very few years ago would have been inconceivable. Who could have guessed that science would progress to the point where we could manipulate genes to forestall disease, transplant cells to halt disease in its tracks and trace age-related diseases to their earliest clues in childhood?

These are just a few of the breakthroughs unfolding on campus, with the promise of many advances yet to come. It’s hard to imagine a more dynamic field than biomedical science, where every finding leads to more insight, more probing, more questions—and ultimately, more answers.

It is a privilege to oversee MCG’s research initiative and to support the faculty, fellows, students and staff who devote their time and passion to pursuing knowledge that will improve the lives of those throughout Georgia and beyond.

It is an exhilarating experience, and one I am proud to share in the following pages. Thank you for taking the time to read about and support MCG’s research.

Sincerely,

Frank A. Treiber, Ph.D.
Vice President for Research, Associate Provost and Regents Professor of Pediatrics
DR. KAPIL BHALLA (LEFT) WITH POSTDOCTORAL FELLOW REKHA RAO
A novel strategy to hopefully beat into oblivion one of the most aggressive forms of acute myelogenous leukemia combines the strengths of some of the newest leukemia agents, researchers say. “These are not traditional chemotherapy regimens. These are targeted therapies that our earlier laboratory studies have shown have a synergistic effect,” said Dr. Kapil N. Bhalla, director of the MCG Cancer Center.

The strategy takes on the mutated protein receptor that enables the deadly proliferation of leukemic cells by degrading it with histone deacetylase and heat shock protein 90 inhibitors. It uses protein kinase inhibitors to reduce the function of any remaining protein and kills off leukemic cells with a natural cell death mechanism called TRAIL.

Dr. Bhalla recently received a five-year, $1.3 million grant from the National Cancer Institute that will enable his research team to do more preclinical testing of the strategy in human leukemic cells and an AML animal model.

About six years ago, researchers found the mutation in the FLT-3 gene that results in the mutated protein receptor on the cell surface. This receptor usually responds to a growth factor that gives rise to normal bone marrow cell proliferation. “But in this case, this mutated protein receptor is constantly triggered, is constantly on and it drives proliferation, promotes survival and shuts down differentiation,” Dr. Bhalla said.

Within weeks, leukemic cells take over the bone marrow, then spread throughout the body. “Patients typically develop abnormalities of white blood cell count and platelet count, anemia or weakness and present with either an infection because they don’t have enough white blood cells or bleeding,” he said.

“We don’t know what causes these mutations, but if you have FLT-3 mutation—about 30 percent of AML patients do—then the leukemia is generally more aggressive,” said Dr. Bhalla. For whatever reason, this aggressive leukemia occurs most commonly in the elderly, so with an aging population, it’s likely to become even more common.

“If you just target FLT-3 with an inhibitor of its activity, that would not be enough,” said Dr. Bhalla. “If you combine it with something that also depletes its levels, that would be better. But if you deplete its levels, inhibit its activity and combine it with another leukemia cell death-inducing agent, it would be even better,” said Dr. Bhalla, who believes the laboratory work will evolve into a strategy that can be used effectively in the clinics, maybe even before the laboratory work is done.

A big plus is that several drugs that do each of these things already are being studied in patients. However, combined effects of these drugs have not been fully studied against leukemia cells, and the drugs just haven’t been used together in patients with leukemia.

For example, one of the histone deacetylase inhibitors Dr. Bhalla will study in the lab, LBHS89, developed by Novartis Corp., he’s also studying in an early clinical trial for patients with leukemia and lymphoma for whom standard therapies have failed. Several FLT-3 kinase inhibitors are under study for a variety of cancers and MCG will soon join one of those studies for leukemia. Apo2L/TRAIL, developed by Genetech, is under study in a variety of solid tumors and leukemia. TRAIL activates on leukemic cells the same death-inducing stimulus immune cells use to kill cancer cells. “It’s a normal mechanism of killing offending cells,” said Dr. Bhalla.

“We have designed combinations of agents that we will be studying in mouse models and against patient-derived leukemia cells. This grant doesn’t fund a clinical trial, but it allows us to take patient samples and study them in vitro to further define why this gene confers poor survival and what combinations can work against it,” said Dr. Bhalla. He notes that since the drugs are new and have not previously been used together,
issues such as unforeseen toxicity will need to be explored.

“We are studying the combination and how it kills, so when the combination goes into the patient, we will be able to get samples from patients, pre- and post-treatment, to see whether what we are observing in the lab works and, if there are patients who still don’t respond, why don’t they?” said Dr. Bhalla.

Histone deacetylase and heat shock protein 90 inhibitors take direct hits at the mutant protein by targeting HSP 90, a molecular chaperone, which, in this case, improperly folds the protein, leaving it active and producing leukemic cells rather than healthy bone marrow cells as needed. Dr. Bhalla’s lab was the first to show the mutant protein kinase is particularly susceptible to depletion by targeting it with HSP 90 or histone deacetylase inhibitors. He also uncovered the synergy of kinase inhibitors.

“We are targeting HSP 90, which folds and keeps this abnormal protein in its active form,” he said. “By using this agent that targets HSP 90, you also take away many other mechanisms that drive cell proliferation and survival. Once you lower the threshold for cell death by depleting this protein, you use additional strategies to kill leukemic cells. It makes it more effective.”

Toni Baker
Selective Targeting

Investigational Drug Capitalizes on Critical Proteins

A drug under study to treat various cancers selectively kills cancer cells because of its affinity for a modified version of a critical heat shock protein they contain, researchers have found.

They found in cancer a modified version of heat shock protein 90, or hsp90, which like most heat shock proteins, promotes cell survival.

They then showed that in breast cancer and leukemia, this modification, called acetylation, confers a strong attraction to investigational drug 17-AAG, said Dr. Yonghua Yang, postdoctoral fellow in molecular oncology in the laboratory of Dr. Kapil Bhalla, director of the MCG Cancer Center.

“17-AAG blocks the activity of hsp90, which normally binds with ATP, an energy source for cells,” said Dr. Yang, who received a training award to present his research at the American Association for Cancer Research Annual Meeting April 14-18 in Los Angeles.

An unfortunate side effect is that 17-AAG also immediately induces hsp70, which can compensate for the cell-supporting activity of hsp90, said Dr. Yang, noting that like hsp90, hsp70 presents a modified form in cancer.

The net effect is that while the drug ably finds its target, to maximize effectiveness it may need to be modified or used in conjunction with another drug to also block hsp70. MCG researchers are in discussions with Novartis and Kosan Pharmaceuticals about how to make one or the other happen.

17-AAG doesn’t seem to care much for normal hsp90 or hsp70 in healthy individuals. Modifications in cancer result from environmental triggers, including stress and eating a lot of oxidized foods, such as foods fried at high temperatures or stored for a long time—more good reasons to relax and eat a well-balanced diet, Dr. Yang said.

The MCG researchers are now looking at the relationship between the modified hsp90 and breast cancer metastasis and developing antibodies that target hsp90.

Heat shock proteins are called molecular chaperones because of their caretaker role. They activate genes that ultimately make proteins, move proteins around cells and fold them into the proper shape so they’ll have the proper function. Chaperones even help proteins group properly and discard old proteins.

Misfolding of proteins, for example, can cause cancer. Molecular chaperones are highly expressed in human cancer and seem resistant to radiation therapy and chemotherapy, according to Dr. Bhalla.

Toni Baker
Better Detector?

Test Targets Most Virulent HPV Strains

MCG researchers are studying a test for the two strains of human papillomavirus responsible for most cervical cancers.

The molecular assay uses a cervical scraping, like that for a liquid-based Pap smear, to test for HPV types 16 and 18, responsible for 70 percent of cervical cancers, said Dr. Daron G. Ferris, MCG family medicine physician and director of the Gynecologic Cancer Prevention Center.

“Data from a National Cancer Institute trial show that if you have a genital infection with HPV types 16 or 18, your chance of getting moderate to severe precancerous cervical changes or cancer is much higher than if you have one of the other types,” said Dr. Ferris, a principal investigator on the national study evaluating the assay.

The NCI study followed women infected with different types of the typically slow-acting virus over 10 years. It found women infected with type 18 had a 15 percent risk of cancerous or pre-cancerous changes after 10 years, those with type 16 had a 20 percent increased risk while those with the 11 other strains had a collective risk of 1-2 percent.

The type-specific assay, developed by Third Wave Technologies, Inc., in Madison, Wis., is being tested along with an assay that detects 14 types of cancer-causing HPV. A test that detects 13 types of HPV already is commercially available, so the new test could become the second non-type-specific HPV test on the market.

Dr. Ferris, who was involved in early studies of the HPV vaccines, hopes the new tests will one day provide better screening options for the most common sexually transmitted disease.

The current national study is giving the new HPV test and the type-specific assay to 1,500 women age 30 and older with a negative Pap test and to 1,000 women age 18 and older with cervical cell changes of undetermined significance—pathologists call this most common abnormal result ASC-US—or higher-grade abnormalities.

“"In the future, we may not do Pap smears on women over 30; it might just be screening with an HPV test. A lot of experts are suggesting that is the way we should go."

—Dr. Daron G. Ferris
“These are the two ways to use HPV testing,” said Dr. Ferris. “One is as a primary screening adjunct test with a Pap test for women age 30 and older and the other is as a triage test when women have an abnormal Pap smear result.”

An HPV test typically follows an ASC-US Pap smear, which at best is about 80 percent accurate.

However, the HPV test, which is more accurate, has not become widely accepted as a primary screening tool for women age 30 and older, Dr. Ferris said, citing cost and the widespread use of Pap smears as likely factors.

“This new HPV test could lengthen the interval of screening for cervical cancer. If the new test is as good as the old one and if the HPV test is negative, there is only a 1 percent chance you have something wrong,” he said, noting that Pap smears really don’t add much to the equation.

Still, when he lectures around the country and asks for a show of hands of physicians using HPV tests in women over 30, few go up; he hopes that will change.

“In the future, we may not do Pap smears on women over 30; it might just be screening with an HPV test. A lot of experts are suggesting that is the way we should go,” he said.

In fact, the American College of Obstetricians and Gynecologists and American Society for Colposcopy and Cervical Pathology already recommend HPV testing in all women over 30.

Younger women are another matter. Ages 15 to 25 are peak sexual activity years and peak years for HPV infection, said Dr. Ferris. Fortunately, the vast majority of the infections are cleared; ones that persist to age 40 are most likely to cause problems. “Seventy percent of patients clear the infection. The 30 percent who don’t, if they still have it by the time they are age 40, are heading down the wrong path,” he said.

Less-expensive Pap smears likely would continue to be used in the under-30 group to catch the few infections that become problematic in this age group.

Currently, there are not FDA-approved drugs to cure cervical pre-cancers and cancers caused by HPV, although centers such as MCG’s are evaluating potential therapies. Resulting cervical changes may be followed with frequent Pap smears or colposcopy, in which physicians can view the cervix and freeze, excise or vaporize significant cellular changes.

Toni Baker

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![Increased Risk of HPV 16 and 18](image)
A jumping gene first identified in a cabbage-eating moth may provide a safer, target-specific alternative to viruses for gene therapy, researchers say.

They compared the ability of the four best-characterized jumping genes, or transposons, to insert themselves into a cell’s DNA and produce a desired change, such as protecting the cell during radiation therapy.

They found the piggyBac transposon was five to 10 times better than the other circular pieces of DNA at making a home and a difference in several mammalian cell lines, including three human ones.

“If we want to add a therapeutic gene, we can put it in the transposon and use it to deliver the gene into the cell,” said Dr. Joseph M. Kaminski, an MCG radiation oncologist and a corresponding author on research published in September 2006 in the online Proceedings of the National Academy of Sciences Early Edition.

“You can use these wherever retroviruses have been used.”

In addition to piggyBac, researchers looked at what was believed to be the most efficient transposon in mammalian cells, hyperactive Sleeping Beauty, first found “asleep” in fish. They also looked at Tol2, another fish transposon, and Mos1, found in insects.

The piggyBac transposon, which has close relatives in the human genome, is widely used to genetically modify insects. Sleeping Beauty has been used to correct hereditary diseases, including hemophilia, in a mouse model.

For this study, researchers used transposons to deliver an antibiotic-resistant gene. They found that while piggyBac might be less efficient than a virus, it puts Sleeping Beauty to shame when it comes to making cells antibiotic-resistant.

“Sleeping Beauty has captured the field as far as transposons to be used in mammals,” said Dr. Stefan Moisyadi, a University of Hawaii molecular biologist and a corresponding author. “But by comparing different transposons, we showed Sleeping Beauty is far inferior to piggyBac.”

Scientists have used viruses to deliver genes for more than 20 years because of their adeptness at infiltrating cells and inserting themselves in DNA. But efficiency comes at a price. Major complications, including deaths, have plagued gene therapy trials.

“With viruses, you don’t have control,” said Dr. Kaminski. “People have tried to modify viruses for site-specific integration and have not been very successful. Once they get into the cell, they can insert wherever they want.”

Dr. Kaminski’s previous work, published in 2002 in The FASEB Journal, hypothesized that the integration site for transposons can be selected.

“Typically, viruses and transposons will integrate anywhere along the genome,” he says. “If they integrate anywhere, it can obviously cause harm. If we can target the integration, be able to insert the gene at a safe spot in the genome, that would be beneficial.” He confirmed that targeting integration is possible in a paper he co-authored in 2005, also in The FASEB Journal.

“We can do it in insects,” said Dr. Moisyadi. “I think it’s a short step to take it to a targeting mechanism we can use in humans.”

Transposons also are cheaper to produce and probably safer than viruses. Another plus: Unlike viruses, they can carry larger genes, such as the dystrophin gene to help correct muscular dystrophy. On the other hand, unlike retroviruses, transposons have to be coated with lipid to slip into cells.

Although piggyBac is not as successful as the virus at integrating into DNA, “we could potentially make a
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hyperactive version of piggyBac, like they did for Sleeping Beauty, which might be as good or better than retroviruses,” Dr. Kaminski said. “I think we’ll do it or somebody will. I think it’s a safer method. One of our next goals is to use transposons to deliver a radio-protective gene, called manganese superoxide dismutase, to potentially protect normal tissue from radiation damage.”

In cancer, he suspects gene therapy will focus on this type of modification of normal tissue for protective purposes as well as manipulating the immune response. However, it has broad applications for correcting single gene disorders, such as hemophilia, sickle cell disease and muscular dystrophy.

Other collaborators include Dr. Sareina Chiung-Yuan Wu, lead author, and Dr. Yaa-Jyunh James Meir, both assistant research scientists at MCG, as well as researchers at Texas A&M University Department of Entomology, the U.S. Department of Agriculture Center for Medical, Agricultural and Veterinary Entomology and the University of Zurich Institute of Laboratory Animal Sciences.

Toni Baker
A little-known lipid plays a big role in turning a hollow sphere of stem cells into a human being, researchers have found.

They found that in the first few days of life, ceramide helps stem cells line up to form the primitive ectoderm from which embryonic tissues develop, said Dr. Erhard Bieberich, an MCG biochemist.

Probably 90 percent of ceramide gathers at the top, or apical, end of these early stem cells, giving cells direction.

“We have cell polarity, an up and down, and that is what ceramide most likely regulates,” said Dr. Bieberich.

“Cell polarity is absolutely essential for differentiation; otherwise, you have a ball of cells, not organized tissue.”

In fact, embryos begin as a wad of cells, but within 24 hours, some cells die and others become part of the hollow sphere with an inner layer—the primitive ectoderm—that will further differentiate into an embryo, and an outer layer—the primitive endoderm—that sustains a fetus.

“Ceramide distributes to the apical end of the cell,” said Kannan Krishnamurthy, an MCG graduate student and first author of the study published in the Feb. 2, 2007 issue of the Journal of Biological Chemistry. “In this case, the basal, or lower, end is attached to the outer layer while the apical end points toward the sphere’s cavity.”

Cells make ceramide, which researchers are finding has many jobs in the developing and mature body. Like other lipids, it helps make up membranes throughout the body, it has an insulation role in the skin and it is a precursor for the protective coating of nerves, called myelin.

“There is more and more evidence that ceramide not only is a structural lipid but a messenger involved in signal transduction, in telling proteins what to do,” said Dr. Guanghu Wang, an MCG research assistant scientist who shares first authorship.

In 2003, Dr. Bieberich and his colleagues reported ceramide teams up with the protein, PAR-4, to eliminate useless cells in developing brains. Now, his team reports that ceramide is vital in

DRS. GUANGHU WANG (FROM LEFT), KANNAN KRISHNAMURTHY AND DR. ERHARD BIEBERICH
establishing cell polarity by attracting certain proteins to the top of the cells, then triggering a series of interactions between them.

When researchers inhibited ceramide production, polarity proteins didn’t gather at the top of cells, cells died and primitive ectoderm formation was impaired. All processes worked like a charm when ceramide was restored. They plan to study ceramide’s potential roles in mature cells and in some cells losing their direction and becoming cancerous.

“There are conditions where a lot of cells die by what we call apoptosis and, in these cases, ceramide may be elevated, causing good cells to die,” said Dr. Bieberich. Ultraviolet radiation, for instance, may increase ceramide levels.

To study the quantity and location of ceramide, the researchers first developed an antibody that binds to it so it could be seen and counted. Previously, chemical studies have documented its presence but nothing more.

Longstanding collaborator Dr. Brian G. Condie, a developmental neurobiologist at the University of Georgia, and Dr. Jeane Silva, an MCG research coordinator, also are study co-authors. The work was funded by the National Institutes of Health.

Toni Baker

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—Dr. Erhard Bieberich
Tailored Treatment

Study Probes Which Breast Cancer Patients Need Chemotherapy

Most postmenopausal women with small breast tumors don’t need chemotherapy to reduce their recurrence risk after lumpectomy.

To try to determine who does, a test that measures a tumor’s aggressiveness based on its DNA will be tested nationally in more than 10,000 of these women.

“Because they have such small tumors, it’s hard to tell who needs chemotherapy,” said Dr. Thomas A. Samuel, an MCG hematologist/oncologist specializing in breast cancer and a study principal investigator.

Of every 100 postmenopausal women with a small tumor that has estrogen receptors and no sign the disease has spread to the lymph nodes, about 12 to 15 need chemotherapy to reduce the risk of recurrence, Dr. Samuel said.

But because no definitive test indicates who needs it, all receive chemotherapy, even though the vast majority would do well with lumpectomy, radiation and hormone therapy that keeps cells from being refueled by estrogen.

Chemotherapy’s potential side effects—including hair loss, nausea, vomiting and increased risk of leukemia and heart problems—are onerous enough to cause some women to discontinue treatment.

“I know that a number of these patients probably don’t need it, but there is no way for me to know who they are ahead of time,” Dr. Samuel said. “I think this trial will help us find who should get it and who should not.”
The commercially available test he is studying—the Oncotype DX™—determines the likelihood that cancer will spread or grow by analyzing 16 tumor genes and five reference genes as controls. “How cancers behave largely depends on what the DNA is like,” Dr. Samuel said.

The test has been on the market more than a year but is expensive and not widely used. Dr. Samuel used it only twice before the study but predicts it will become a standard part of treatment if the federally funded study supports current findings.

Dr. Samuel said. Most will be randomized to either get chemotherapy or not.

Dr. Samuel hopes to enroll about two patients per month; the trial likely will be open several years depending on how long it takes to enroll 10,000 women nationally. Participants will be followed for at least five years.

Toni Baker
Veterans exposed to Agent Orange have a 48 percent higher risk of prostate cancer recurrence following surgery than their unexposed peers, and when the disease comes back, it seems more aggressive, researchers say.

“We need to be screening these patients earlier, treating their cancer aggressively and following them closely afterward,” said Dr. Martha Terris, chief of the Urology Department at the Augusta Veterans Affairs Medical Center and an MCG professor of urology.

“We looked at all patients, whether they were exposed or not, to see which were more likely to develop a recurrence, and patients with a history of Agent Orange exposure were more likely,” said Dr. Sagar R. Shah, an MCG urology resident who presented the data during the American Urological Association Annual Meeting in May 2007.

The study looked at 1,653 veterans who had prostate cancer surgery at Department of Veterans Affairs Medical Centers in five cities between 1990 and 2006; 199 had been exposed to Agent Orange, a herbicide and defoliant sprayed on the dense forests of Vietnam during the war.

Agent Orange contains the carcinogen, dioxin, which can be stored in body fat and is believed to penetrate the cell nucleus and work as a tumor promoter. Relatively higher mortality rates have been found in chemical plant workers and farmers with prostate cancer who were exposed to dioxin, the researchers wrote in their abstract.

Researchers found veterans with Agent Orange exposure were disproportionately African-American and younger than average to have a cancerous prostate gland removed. (They suspect that African-Americans, who were more likely to be ground troops during the Vietnam War, were at increased risk for higher levels of exposure.)

Echoes from the Past

Agent Orange Exposure Heightens Cancer Recurrence Risk

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The disease appeared to be caught earlier in exposed veterans. Most had their disease staged as T1 (seemingly confined to the prostate gland) and had lower pre-operative prostate specific antigen scores, an indicator of disease aggressiveness.

However, when the disease recurred, exposed veterans experienced a more rapid biochemical progression of their disease, which PSA measures. In African-Americans, the PSA doubled in almost half the time of their unexposed peers.

A blood PSA level screens for prostate cancer for most men beginning at age 50 and at age 40 for blacks and men with a family history. Black men have been shown by Dr. Terris and others to have more aggressive disease earlier in life.

To account for known racial differences, researchers also compared recurrence rates in exposed and non-exposed blacks and whites. The results held up.

“As a population in general, if you were exposed to Agent Orange, you’re more likely to have a recurrence,” says Dr. Shah. “If you are black and were exposed, your cancer is more likely to recur than if you are black and unexposed.”

The study was funded by the Georgia Cancer Coalition and the Department of Veterans Affairs.

Toni Baker
Bak-Up Plan

Protein Seems to Doom Stressed Cells to Suicide

When a cell is seriously stressed, say by a heart attack, stroke or cancer, a protein called Bak just may set it up for suicide, researchers have found.

In a deadly double whammy, Bak helps chop the finger-like filament shape of the cell’s powerhouse, or mitochondrion, into vulnerable little spheres. Another protein, Bax, then pokes countless holes in those spheres, spilling their pro-death contents into the cell.

“We found out Bak has a distinct function in regulation of the mitochondrial morphology,” said Dr. Zheng Dong, a cell biologist at the Medical College of Georgia and the Veterans Affairs Medical Center in Augusta and corresponding author on a paper published recently in Proceedings of the National Academy of Sciences. “Bax, on the other hand, is not involved in morphological regulation but needs to be there to puncture holes.”

“One has to break up, kind of soften, the mitochondria for injury, and the other one actually punches the holes to kill it,” said Craig Brooks, MCG graduate student and the paper’s first author.

Bak and Bax have similar structures, and scientists have long suspected they play major, similar roles in programmed cell death, or apoptosis. “These two proteins are very important for mitochondrial injury and subsequent apoptosis,” said Dr. Dong.

To stress cells, they blocked oxygen supplies and used the common chemotherapeutic agent cisplatin, then documented that filamentous mitochondria became fragmented very early and quickly in apoptosis. Ironically they also found the deadly fragmentation results from Bak’s interaction with mitochondria-shaping proteins called mitofusins, which help mitochondria keep their filamentous shape in non-stressed cells. Dr. Dong suspects Bak may also play a role in mitofusin regulation in normal, non-stressful conditions.

In fact, the researchers suspect Bak, Bax and the contents they spill into the cell all have roles in keeping a cell functioning until a stressor kicks in.

“They probably are both kept in check normally in the cell by other proteins, and when something happens that overwhels the cell, it activates Bak and Bax to start cell death,” said Mr. Brooks.

“Some of the same proteins, cytochrome c is the big one, are needed for daily mitochondrial function like making energy, but if they are released from the mitochondria, they activate a cell-killing or apoptotic pathway,” said Dr. Dong, referencing the contents that spill from punctured mitochondria.

Looking at kidney cells and neurons in a Bak-deficient mouse, they also showed that Bak and Bax need each other to successfully spawn cell suicide. “If you have Bak but not Bax, the mitochondria still fragment but they don’t die; if you have Bax but not Bak, you still have punctures in the mitochondria but with low efficiency,” said Mr. Brooks.

Now they want to know exactly how Bak interacts with mitofusins, how the interaction is regulated and how it affects mitochondrial morphology, physiology and pathology. Their long-term goal for better understanding the cell suicide mechanism is developing drugs to block it in the case of a stroke, for example, or induce it to kill cancer.

Dr. Dong recently received a $1.1 million, four-year renewal grant from the National Institute of Diabetes & Digestive & Kidney Diseases to further study the structural changes of mitochondria during apoptosis and normal physiological conditions.

Toni Baker
We found out Bak has a distinct function in regulation of the mitochondrial morphology. Bax, on the other hand, is not involved in morphological function but needs to be there to puncture holes.

—Dr. Zheng Dong
Knowledge is Power

Web-Based Program Lays Out Treatment Options

A Web-based program that informs prostate cancer patients about treatment options may ease the decision of which to choose, MCG researchers say.

Treatments for localized prostate cancer and associated side effects are so varied that patients are often confused about which option is best for them, said Dr. Gerald Bennett, chair of the Department of Health Environments and Systems in the MCG School of Nursing.

“This disease can be treated by observation alone, surgery, cryosurgery, hormonal therapy and radiation therapies,” Dr. Bennett said. “But there have only been a few studies that adequately compare the complications of different treatments, which can include sexual, bladder and bowel dysfunction. Men can hear their doctors’ recommendations, but ultimately, they decide which treatment to pursue. Those decisions can dramatically affect their lives, but the bottom line is that we often don’t know enough scientifically to recommend one treatment over the other.”

MCG is part of a National Institutes of Health-funded study to determine the impact of the Personal Patient Profile Prostate (P4) program, an innovative computer program that measures personal factors and creates an Internet decision-support system.

Led by the University of Washington in Seattle, the other sites are Fox Chase Cancer Center in Philadelphia and the University of Texas Health Science Center in San Antonio. Nearly 500 patients will be included in the study nationwide.

In Augusta, Dr. Bennett and his research team will recruit 72 prostate cancer patients from the Augusta Veterans Affairs Medical Center. Half will follow a traditional treatment plan—diagnosis followed by a consultation with a cancer specialist and treatment—and half will use the P4 program.

“These men will go to the Web site and answer questions like who they feel should be responsible for making treatment decisions—their doctor, themselves or a combination of the two—and the program will provide video examples of how to approach those discussions with their care providers,” Dr. Bennett said. “We believe men who have access to the P4 program will have less inner conflict while making treatment decisions and, in the long run, will be more satisfied with whatever treatment path they choose.”

“I often see patients struggle with treatment decisions,” added Dr. Martha Terris, urologist at the Veterans Affairs Medical Center in Augusta and MCG. “While a program like this one doesn’t make the treatment decision for them, it does help them make better-informed decisions and further open the lines of communication with their doctors.”

Jennifer Hilliard

Drs. Rosalind Jones (left) and Gerald Bennett
Mounting evidence suggests that inflammation, a part of the immune response implicated in diseases such as cancer, Alzheimer’s and diabetes, may also help translate stress into high blood pressure.

“There is a concept that hypertension is an inflammatory condition,” said Dr. Haidong Zhu, an MCG molecular geneticist who believes the connection lies in the kidneys’ ability to release sodium.

When stress activates the sympathetic nervous system (the fight-or-flight mechanism), the body increases production of interleukin 6, a pro-inflammatory factor, which ultimately leads to production of other inflammatory factors such as C reactive protein.

Stress also prompts the body to retain sodium to help temporarily raise blood pressure, said Dr. Gregory Harshfield, director of MCG’s Georgia Prevention Institute and an expert on what happens when the body doesn’t let go afterward. He has documented the condition, called impaired stress-induced pressure natriuresis, in otherwise healthy teens.

Dr. Zhu suspects the condition may be caused by mutations in four sets of stress-activated inflammatory genes: interleukin 6, interleukin 6 receptor, cytokine signal transducer and C-reactive protein.

Her research team, funded by a two-year, $300,000 National Heart, Lung and Blood Institute Grant, is seeking variations of the genes in 500 teens with normal blood pressure. The teens, already enrolled in GPI studies measuring the effects of stress on the body, will undergo DNA tests.
cardiovascular system, were put on a diet for four days to regulate sodium intake, then came to the GPI where they rested for an hour, played a racing video game for an hour, then rested for an hour.

Blood and urine samples were taken throughout the period. For this study, researchers will also collect DNA material from as many parents as possible to confirm their findings.

Pilot data indicate that black teens with normal blood pressure and a certain variation of the interleukin 6 gene have significantly reduced sodium excretion in the urine following stress.

Researchers have further implicated the inflammatory factor’s role in blood pressure regulation by showing that following stress, circulating levels of interleukin 6 rise and are still up an hour after the stressor is gone.

Dr. Zhu suspects that even without the genetic variations, inflammation affects blood pressure under stress, so she will study its impact alone and in concert with the mutations.

“Our long-term goal is to be able to identify a subgroup of individuals with a certain genetic profile that has an increased risk of developing high blood pressure in a stressful environment,” said Dr. Zhu.

If successful, those with salt-sensitive hypertension—which includes approximately half of Americans with high blood pressure—might benefit from a low-salt diet, exercise and anti-inflammatory drugs.

The relationship between inflammation and high blood pressure, including how cytokines affect blood pressure, also is the subject of a five-year, $11 million National Heart, Lung and Blood Institute Program Project study at MCG led by Dr. R. Clinton Webb, chair of the Department of Physiology.

“"Our long-term goal is to be able to identify a subgroup of individuals with a certain genetic profile that has an increased risk of developing high blood pressure in a stressful environment."”

—Dr. Haidong Zhu
Salt Assault

Study Seeks Genetic Predictors of Hypertension

MCG researchers are studying the rare disorder, Liddle syndrome, in search of genetic risk factors for hypertension.

The disease was first reported in 1963 in a teen with high blood pressure. Interestingly, an inexpensive diuretic worked best to manage her problem. Tests years later found the reason was that genes involved in the channel that recycles sodium from food into the body were drastically mutated. “This mutation enables sodium to come back into the body like a flood,” said Dr. Yanbin Dong, an MCG molecular geneticist and cardiologist.

He is looking at sodium channel genes implicated in Liddle syndrome to identify subtler changes that could be used to screen for hypertension risk in the general population.

“My hypothesis is if Liddle syndrome is caused by these nasty, drastic mutations, maybe the majority of hypertension can be caused by milder, less nasty polymorphisms or variations in the same genes,” said Dr. Dong, who received a $1.43 million grant from the National Heart, Lung and Blood Institute to test his theory.

He is recruiting 300 healthy black teens with normal blood pressure for a Georgia Prevention Institute study that first measures sodium-handling following environmental stress, then analyzes the genes of those who don’t handle it well.

Dr. Dong is exploring findings by Dr. Gregory A. Harshfield, director of the Georgia Prevention Institute, that some healthy youths continue to retain sodium following stress. He documented this impaired stress-induced pressure natriuresis in about 36 percent of healthy black youths and 25 percent of healthy white youths.

Blood pressure naturally rises during stress, immediately by constricting blood vessels and longer-term by directing the kidneys to retain more sodium and so increase blood volume, said Dr. Harshfield, a co-investigator on Dr. Dong’s latest grant. His own studies have shown the importance of the interaction between salt and stress in regulating blood pressure.

The new study should provide additional insight into the relationship between salt and stress as well as diet and genetics, Dr. Dong said.

Study participants will be on a salt-restricted diet for four days, then come to the GPI on the fifth day to rest for an hour, play competitive video games and rest again. Blood pressure and sodium excretion will be measured before games are played, immediately afterward, then two hours later.

The five genes—alphaENaC, betaENaC, gamma ENaC, SGK-1 and Nedd4-2—will be analyzed from blood samples so specific variations can be correlated with the ability to excrete sodium following stress. Gene-to-gene interactions also will be studied.

If Dr. Dong’s hypothesis holds, young people with genetic risks for hypertension could have advance warning and make changes—such as a low-sodium diet and stress management—to ideally avoid the problem.

Toni Baker
By age 10, some black children already have high nighttime blood pressure, an early predictor of cardiovascular disease, a new study shows.

As they grow up, black children also show greater increases in nighttime blood pressure, according to a study that followed children’s blood pressures over 15 years.

Blacks experience less of a dip in nighttime blood pressure than whites. The gap between the pressure measurements of whites and blacks also widens as children get older.

At night, blood pressure should drop because the body is resting, said Dr. Gregory Harshfield, director of MCG’s Georgia Prevention Institute and a co-author on the study published in the Dec. 19, 2006 edition of Circulation, the journal of the American Heart Association.

One reason for the higher nighttime pressure is that some blacks retain more sodium, which increases fluid volume in their bodies and their blood pressure, according to researchers.

To determine pressure differences between black and white children and at what age those differences occur, Dr. Frank Treiber, vice president for research and study co-author, measured the ambulatory blood pressures of almost 700 children 12 times during 15 years.

“Most previous studies have looked at a cross-section of people, and this 15-year study allows us to look at one population over an extended period of time,” said Dr. Xiaoling Wang, genetic epidemiologist and the study’s lead author. “This helps us identify the age these problems begin to occur—as early as age 10.”

Ethnic differences were measurable in study subjects even after researchers controlled for factors such as height, body mass index, socioeconomic status and stress-related coping strategies.

Interestingly, she said, tests of people of African descent who live outside the United States have shown normal nighttime blood pressure.

“That most likely means that the problem is not purely genetic and also is likely caused by environmental factors like salt intake and stress,” Dr. Wang said. “It has already been proven that stress causes sodium retention, but there could be also other factors. This study supports existing research and warrants further study of the causes for racial differences in blood pressure at an early age.”

Study co-authors included Dr. Coral Hanevold, a pediatric nephrologist; Dr. Joseph Poole, an MCG student; and Dr. Harold Snieder, an MCG biostatistician.

Jennifer Hilliard
While men and women both get high blood pressure and related kidney disease, the path to get there is shorter, steeper and just different for men, researchers say.

“They may end up at the same point, but the way they got there could be very different,” said Dr. Jennifer C. Sullivan, a pharmacologist/physiologist at MCG’s Vascular Biology Center.

“It’s known that men tend to develop hypertension earlier than women and the increase in blood pressure occurs more rapidly than it does in women, until they hit menopause. I look at our spontaneously hypertensive rats and see the same dichotomy in blood pressure,” Dr. Sullivan said of the animal model she studies.

“There are also differences in development of renal injury in the human population, and chronic renal disease seems to be worse in men. I see the same thing in my animal model.”

Dr. Sullivan, who recently received the 2007 New Investigator Award of the American Physiological Society’s Water and Electrolyte Homeostasis Section, is studying these gender differences to learn what protects pre-menopausal females.

Female hormones can’t account for all the difference, she said. “It’s not that easy. Men and women are more than just sex hormones.” When the rats’ testicles are removed, for example, blood pressure and injury incidence drop slightly; when ovaries are removed, blood pressure remains unchanged but kidney injury increases slightly.

“There are fundamental differences, I believe, in the physiology. They are going to end up at the same point, but the way there could be very different.”

She is comparing two major players in hypertension—vasoconstriction and levels of free radicals—in males and females.

She is finding that nitric oxide synthase (a molecule that signals smooth muscle cells to relax) may make more nitric oxide in females. Just how active an enzyme is depends on how it is
phosphorylized, or turned on by adding phosphate groups. “Our preliminary data say that the phosphorylation status may increase nitric oxide production—and maintain kidney health—in females,” Dr. Sullivan said.

In contrast is the powerful constrictor of blood vessels, angiotensin 2. Males’ outer kidneys (the renal cortex) have ample AT1 receptors, enabling angiotensin 2 to do harm. “It’s a vasoconstrictor when it binds with AT1,” she said. “It will cause proliferation and hypertrophy, it can stimulate the production of reactive oxygen species, so it does all sorts of bad things.”

Fortunately, drugs are available to block angiotensin 2’s destructive action: angiotensin receptor blockers and ace inhibitors. Interestingly, clinical studies already have shown these drugs don’t work as well in women. “A lot of women are on these drugs, and I’m not sure it’s doing them a lot of good,” she said. One of her goals is to find out.

Males also have too many highly reactive and potentially damaging free radicals in the renal cortex. Free radicals or reactive oxygen species have important jobs in the body, but an excess creates oxidative stress, a contributor to most major diseases. In the case of high blood pressure, free radicals damage proteins critical to blood vessels and kidneys.

“The body has natural mechanisms for keeping free radicals in check, including endogenous antioxidants. “But if you get increases, it can overwhelm the natural ability of the body to take care of it,” she said.

When she looks at the toll on the kidneys, she finds about a 50 percent increase in the amount of protein excreted in the urine—a sure sign of kidney disease—in the males.

She notes that by age 70, the rates of cardiovascular disease and hypertension are similar in men and women and that older women tend to have higher blood pressures than age-matched men.

Her work is funded in part by an American Heart Association Scientist Development Grant.

Toni Baker
How drugs such as adrenaline do primarily one thing—in this case increase the heart rate—now makes more sense to scientists.

“Any time you get a sudden jolt, adrenaline is why your heart rate goes up,” said Dr. Nevin A. Lambert, an MCG biophysicist.

Research published in the Nov. 21, 2006 issue of Proceedings of the National Academy of Sciences may help explain how cells respond correctly to adrenaline, or epinephrine.

Most drugs never penetrate cells; they interact with external receptors that activate G proteins inside cells. “It’s like a relay,” said Dr. Lambert. “G proteins collide with receptors. The receptor itself does not do anything other than turn on these G proteins.”

There are only four classes of G proteins, but cells contain thousands of copies of them which interact with hundreds of surface receptors. Each G protein is actually three protein subunits stuck together: alpha, beta and gamma.

Textbooks teach that once G proteins are activated, the alpha protein splits from the beta and gamma subunits, which are irrevocably stuck together as a beta-gamma pair. Each half of the now dissociated G protein can cause the cell to do something different. “Sometimes they help each other out; sometimes they work at cross purposes,” said Dr. Lambert.

With epinephrine, that should mean the alpha subunit enables production of cyclic AMP, which increases the heart rate, while the beta-gamma pair should activate ion channels, making cells less electrically excitable and decreasing the heart rate.

However, it has been known for some time that while epinephrine does increase cyclic AMP in heart cells, it does not activate ion channels. It has been unclear how the cell allows one response and suppresses the other.

The answer likely is because the G proteins activated by epinephrine receptors don’t readily dissociate, contrary to the textbook picture. MCG researchers have also shown that at least one other class of G proteins does dissociate, suggesting the textbook picture is at least partly correct.

Why the difference? Previous work on G proteins, including their discovery and studies of their role in signal transduction, was mostly done in test tubes using purified proteins. MCG researchers used a technique they developed to actually look at G protein function inside living human cells.

Their findings suggest that epinephrine interacts with a G protein that doesn’t release the beta-gamma subunit.

“There was a constant question about how drugs sometimes avoid doing unwanted things,” said Dr. Lambert. “This helps us understand how drugs can be specific. The flipside of the coin is some drugs acting on some receptors will have multiple actions because the G proteins do dissociate.”

The newfound information is no doubt only one step toward better understanding how hundreds of receptors can act through just four classes of G proteins and produce so many physiologic results. “It’s like 100 cars driving down four roads and ending up in 100 different places,” Dr. Lambert said.

But it’s a timely finding as science moves toward designer drugs, including some that might target G proteins directly, bypassing intermediary receptors, with the hope of getting a more robust response.

In Dr. Lambert’s lab, MCG graduate student Gregory J. Digby, first author on
the *PNAS* paper, is now looking at G protein subunits that do and don’t fall apart with the long-range goal of designing ones that do what the researchers want.

“Right now, it’s all engineering for the sake of understanding how they work,” said Dr. Lambert.

Researchers suspect the stickiness between the subunits determines whether they split, and that the bottom line will be two classes of G proteins dissociating and two not.

Other co-authors include Robert M. Lober, M.D./Ph.D. student, and Pooja R. Sethi, laboratory technician. Dr. Alfred G. Gilman, longtime chair of pharmacology and now dean of the University of Texas Southwestern Medical School who won the 1994 Nobel Prize in Medicine for discovering G proteins, edited the paper.

The research was funded by the National Science Foundation and the National Institutes of Health.

*Toni Baker*
Faulty HDL, Lifestyle Linked to Asians’ Risk of Heart Disease

South Asian immigrants in America have a higher risk of heart disease than any other American population. The trouble, MCG researchers suspect, is a combination of dysfunctional high-density lipoprotein (the so-called “good” cholesterol) and a Westernized lifestyle.

“Research shows that while only 9 percent of whites develop coronary artery disease, between 18 and 25 percent of South Asian immigrants eventually develop it,” said Dr. Sunita Dodani, an MCG epidemiologist, cardiologist and assistant dean for research in the School of Nursing. “Interestingly, South Asians who live in their homelands have normal rates of the disease.”

In most South Asians, dysfunctional HDL, first identified by the historic Framingham Heart Study, is likely caused by a mutation of Apo-A1, the gene that codes the major protein component of HDL, Dr. Dodani said.

“HDL can only protect people from heart disease if it’s functional,” she explained. “The dysfunctional HDL and external risk factors like stress from moving and new jobs and high-fat diets make for a deadly combination.”

Dr. Dodani, a South Asian immigrant herself, and collaborators at MCG and the University of California at Los Angeles have been studying 29 Augusta immigrants, seeking a connection between the gene mutation and dysfunctional HDL.

Blood samples were sent to UCLA to determine whether each subject had dysfunctional HDL. DNA sequencing helped researchers target Apo-A1 mutations, and a portable carotid Doppler machine measured the thickness of carotid arteries.
“Recent research has shown that thickening of the carotid arteries is directly related to thickening of the coronary arteries,” said Dr. Dodani. “The Doppler machine is less invasive than angiography (injecting a contrast medium into a patient) and can just as easily detect the disease in the early stages.”

Dr. Dodani and her colleagues suspected that subjects with thickened carotid arteries would have dysfunctional HDL and a polymorphism of the Apo-A1 gene. This is the first time researchers have examined a possible correlation between the gene polymorphisms, dysfunctional HDL and arterial thickness.

Interestingly, 40 percent of the study population had arterial thickness and, among that group, 17 percent had high blood pressure and more than 30 percent had high cholesterol. Half the study population also had dysfunctional HDL. DNA analysis of those blood samples also found six different mutations of Apo-A1.

“This is a strong indicator that these novel polymorphisms, which haven’t been found in any previous studies, are linked to dysfunctional HDL,” Dr. Dodani said. “These findings support our theory that the disease in South Asians is most likely caused by a combination of dysfunctional HDL, a stressful lifestyle and high-fat diets.

That could explain the high rates in immigrants and not those who stay in their homelands.”

Dr. Dodani has applied for National Institutes of Health funding to enlarge the study and eventually include other populations for comparison.

“Then we will be able to figure out what’s next,” she said. “If the cause is a combination of this gene mutation and other factors, are there preventive strategies that we can employ? Should cholesterol-lowering drugs be started earlier in certain populations? Those are questions we hope to eventually answer.”

Jennifer Hilliard

Research shows that while only 9 percent of whites develop coronary artery disease, between 18 and 25 percent of South Asian immigrants eventually develop it.

—Dr. Sunita Dodani
An interdisciplinary MCG team is probing whether exposure to secondhand smoke increases the chance that children with a family history of cardiovascular disease will develop the disease.

If those children also have a variation in at least one of four genes responsible for metabolizing nicotine, their risk may increase even more because nicotine might stay in the body longer and do more damage.

The team will study 585 people age 15-20 who have a parent, grandparent or both with essential hypertension and/or a heart attack by age 55.

“What I hope to take away from this is more information for parents and caregivers about the risk of future disease that their behavior places on their child,” said Dr. Martha Tingen, a nurse researcher at MCG’s Georgia Prevention Institute and principal investigator on the $220,000 National Institute of Nursing Research grant.

Researchers will look for adverse clinical cardiovascular measures, including reduced ability of arteries to dilate; the blood encountering increased resistance as it travels through vessels; higher blood pressure; and an increase in the size of the pumping chamber of the heart—a result of pumping against elevated pressure.

Exposure to the damaging effects of nicotine and other pathogens in smoke may also cause a vicious cycle in the body. “It likely damages cells on the inner-wall lining of blood vessels, which results in less adaptive capacity of the vessels and arteries. This may cause greater strain on the heart,” said Dr. Tingen.

Children exposed to secondhand smoke who have a variation of one or more of the genes that metabolize nicotine—CYP1A1, GSTM1, GSTT1 and CYP2A6—can experience cellular damage because the nicotine does not leave the body as
quickly, she said. “And if that’s happening, they’re going to have more of these adverse preclinical cardiovascular measures that predispose them to developing cardiovascular disease.”

Almost half a million Georgia children are exposed to secondhand smoke in the home, and up to 60,000 cardiovascular deaths each year in the United States are linked to non-smokers’ exposure to secondhand smoke, Dr. Tingen said.

The researchers will unfreeze the blood samples, which come from a 15-year longitudinal database kept by Dr. Frank Treiber, MCG vice president for research and a GPI child psychologist. Then Drs. Yanbin Dong and Haidong Zhu, MCG molecular geneticists, will seek variations in the identified genes. Dr. Gaston Kapuku, a GPI cardiologist, will help interpret the cardiovascular measures. Finally, the blood samples will be analyzed for cotinine levels, a metabolized version of nicotine and a reliable indicator of secondhand smoke exposure.

“If kids are exposed in the home and they have genetic alterations that make nicotine stay in the body longer, there’s an increased likelihood they’re at greater risk for developing cardiovascular disease,” said Dr. Tingen.

Jennifer Hilliard

“[Exposure to smoke] likely damages cells on the inner-wall lining of blood vessels, which results in less adaptive capacity of the vessels and arteries. This may cause greater strain on the heart.”

–Dr. Martha Tingen
The Toll at Home

Wartime Raises Stress in Military Offspring

Children with parents in the military have higher blood pressure, heart rates and general stress levels than their peers during wartime, researchers say.

Researchers looked at 121 adolescents—48 with civilian parents, 20 with a parent deployed to Iraq and 53 with a parent in the military but not deployed—days after Operation Iraqi Freedom was launched in March 2003 and three months later when President Bush announced major hostilities had ceased.

At both points, adolescent offspring of military personnel self-reported higher levels of stress, reports that were supported by blood pressure and heart rate measures.

“We expected stress levels would push up blood pressure and heart rates,” said Dr. Vernon Barnes, an MCG physiologist and principal author of a paper published in the January 2007 issue of Military Medicine.

Dr. Barnes and his colleagues used a posttraumatic stress disorder questionnaire, psychosocial survey and physiological data to assess stress levels of the teens, who were students at Augusta’s Academy of Richmond County.

Acknowledging that the study was small and did not assess non-war-related stressors, the researchers believe the results merit attention. “We are not aware of any [other] studies examining the impact of the onset of the war on both stress levels and blood pressure of military offspring,” Dr. Barnes said.

There is evidence of the impact of environmental stress on blood pressure and heart rate, important indicators of cardiovascular health, he said. “Certainly, the stress response is increased in soldiers, but this research indicates that it’s also increased in the families they leave behind.”

“Given the continued presence of U.S. soldiers deployed to [Operation Iraqi Freedom] and mounting casualties, these findings suggest that youth with family members in the military, particularly those deployed overseas, may warrant increased attention of parents, educators and counselors during this period of active conflict,” the researchers wrote. “Further research is warranted to determine whether stress reduction interventions may be effective in reducing stress levels and associated indices of sympathetic nervous system arousal in children of military personnel.”

The work was funded in part by the National Heart, Lung and Blood Institute; a research abstract was presented at the American Psychosomatic Society’s 2004 annual meeting.

Toni Baker
An MCG bioinformatics expert is coordinating a national effort to develop animal models to study diabetes complications.

Dr. Richard A. McIndoe, associate director of the MCG Center for Biotechnology and Genomic Medicine, has received a $15 million, five-year grant—MCG’s largest ever—to continue operating the Coordinating and Bioinformatics Unit for the innovative National Institutes of Health project, Animal Models of Diabetic Complications Consortium.

He also will begin providing the same services for the Mouse Metabolic Phenotyping Centers, another NIH-funded consortium of centers offering mouse-testing expertise to scientists nationwide for diseases including diabetes, obesity and related disorders.

The Animal Models of Diabetic Complications Consortium consists of 13 investigators generating ideas for mouse models, a Mouse Generation and Husbandry Core to generate the mice and the Coordinating and Bioinformatics Unit to oversee consortium activities.

“The NIH recognized years ago that there were few good animal models that mimic the complications of diabetes,” Dr. McIndoe said. Even the NOD mouse, a spontaneous model for type 1 diabetes, is inadequate, primarily because complications tend to come with age and mice have a relatively short lifespan.

Diabetes complications include cardiovascular and kidney disease, diabetic retinopathy and nerve and bladder damage. “Diabetic cardiovascular disease is probably the biggest mortality risk for type 1 and 2 diabetes; somewhere around 60 to 70 percent of diabetic mortality can be associated with cardiovascular disease,” Dr. McIndoe said.

The high risk of model development impeded financial support until the NIH committed funds several years ago. Scientists who receive funding agree to make their development data and resulting animal models available to the scientific community.

In 2001, while on the University of Florida faculty, Dr. McIndoe received the first grant to provide administrative and coordinating activities for investigators working on model development. Work includes organizing semi-annual Executive Steering Committee meetings, monthly teleconferences, workshops, training sessions and organizing activities for the External Advisory Boards.

A major task was developing a computer system that could store and analyze the huge amount of data generated by investigators, then share it
with scientists all over the world through a Web portal, www.amdcc.org.

“We have to have a way of storing and capturing all that information in an efficient way so another researcher can go back and do the same experiment or analyze it in real time,” Dr. McIndoe said. “You also need to store information in a way that is very flexible so they can grab the information any way they want. We are constantly adding statistical analysis so data can be analyzed quicker.”

To date, about 70 animal models have been studied, information on about 25 has been deposited in the database Dr. McIndoe developed, and about 20 of those models will soon be available from mouse repositories. Scientists will generally need several models to mimic human disease.

“They don’t want an animal model that looks like a mouse problem; they want an animal model that looks like a human problem,” Dr. McIndoe said.

For the second round of NIH funding, each investigator will propose two new models and turn them over to a husbandry core for development. “Once created, the models will be sent back to the investigators, who will be in charge of understanding the pathology of the complications,” said Dr. McIndoe. The NIH integrated operation of the consortium with the Mouse Metabolic Phenotyping Centers, which also were

up for grant renewal. The centers’ first round of funding didn’t include money for administration and bioinformatics, but it was quickly determined both were needed.

“The centers bring to the general scientific community a low-cost way of doing a variety of metabolic assays on mice that would be cost-prohibitive to set up in your local lab,” Dr. McIndoe said. For a small fee, centers will characterize mouse metabolism, blood components including hormones, energy balance, eating and exercise, organ function and form, physiology and histology. The University of Cincinnati, Vanderbilt University and the University of Washington have been designated as Mouse Metabolic Phenotyping Centers. MCG’s Coordinating and Bioinformatics Unit is soliciting additional centers, which will be funded through a subcontract with MCG, Dr. McIndoe said.

The Animal Models of Diabetic Complications Consortium and the Mouse Metabolic Phenotyping Centers will continue to function autonomously. But Dr. McIndoe has gutted the infrastructure he created for the consortium to accommodate the workings of both. “The face of it will be individual, but the underlying software architecturally works together.”

“This grant, the largest award ever received by MCG, is on target with the NIH’s initiatives to accelerate translation of scientific discoveries into improved health care,” said MCG Vice President for Research Frank Treiber.

“The grant will greatly strengthen our external competitiveness for other center grants,” said MCG School of Medicine Dean D. Douglas Miller. “It also will help our internal planning efforts in the area of data coordination for clinical translational research, a major strategic focus of the school.”

At MCG, Dr. McIndoe also is the local director of informatics for two major newborn screening studies for type 1 diabetes and co-principal investigator on studies seeking type 1 diabetes biomarkers.

Toni Baker

FOR INFORMATION ABOUT MOUSE METABOLIC PHENOTYPING SERVICES AND FEES, VISIT www.mmpc.org.
Teens who are most physically active and consume the most calories are the leanest, researchers say.

“The take-home message is to encourage your child to do as much physical activity as possible, including at least one hour of moderate to vigorous physical activity daily,” said Dr. Paule Barbeau, an MCG exercise physiologist and corresponding author on the paper in the April issue of The International Journal of Obesity. “This allows your child to eat more calories, which encourages more healthy eating habits while remaining in energy balance.”

Unfortunately, even the leanest of the 661 healthy Augusta teens in the study lacked good eating habits, researchers noted.

In fact, researchers couldn’t compare the diet quality of leaner and chubbier teens because it was so poor overall, said Inger Stallmann-Jorgensen, research dietitian and the paper’s first author.

“Most did not have enough whole-grain food, low-fat dairy products, fruits or vegetables,” Ms. Stallmann-Jorgensen said. Dietary mainstays included starches, salty snacks and soft drinks.

“Eating habits formed during our youth tend to stay with us into adulthood, so this does not bode well for prevention of chronic diseases such as diabetes and heart disease,” Ms. Stallmann-Jorgensen noted.

Researchers queried participants about their physical activity and diet over at least four 24-hour periods and calculated body fat percentages. They performed magnetic resonance imaging exams on most participants to measure visceral adipose tissue.

“Eight- to 12-year-olds can have enough that it’s more highly correlated with cardiovascular risk factors than overall percent body fat,” said Dr. Barbeau, noting that even relatively thin children can have enough visceral fat to be a health problem.

Surprisingly, they found teens who ate the most tended to have the least visceral body fat, probably because of their higher activity levels. Conversely, some teens who ate the least had the highest percentage of body fat.

“We expected the energy intake to be lower in kids who were leaner, but we realized the leaner kids were at a different energy balance than the others,” Dr. Barbeau said.

On average, female study participants had 30 percent body fat (high for females) and males had a healthier 18 percent. Genetics also plays a role in the body fat equation, researchers noted.

About 36 percent of high-school students—mostly males—meet recommendations for daily physical activity, according to the Centers for Disease Control and Prevention. On school days, 21 percent of students play video games or use the computer recreationally three or more hours, and 37 percent watch three or more hours of television, a CDC survey showed.

In the MCG study, common teen activity included watching a movie or spending time with friends. The most physically active teens tended to be males who participated in organized sports or exercises they could do alone or with friends.

Parents can help improve their children’s habits by improving their own eating and exercise habits, the researchers agreed. “Children will follow examples set by parents and other caregivers,” Ms. Stallmann-Jorgensen said.

The research was funded by the National Institutes of Health.

Toni Baker
Resting E-Zzzzz

Study Shows Benefits of Exercise on Children’s Sleep

One-fourth of overweight children may have sleep problems that regular physical activity can largely resolve, researchers say.

Research published in the November 2006 issue of *Obesity* shows a surprising 25 out of 100 overweight, inactive children tested positive for sleep-disordered breathing, including telltale snoring.

After about three months of vigorous after-school exercise, the number of children who tested positive for a sleep disorder was cut in half, according to Dr. Catherine L. Davis. In children who exercised the longest, the number was reduced by 80 percent.

The children were among 100 boys and girls age 7 to 11 enrolled in a study of the effects of exercise on metabolism. For that study, the children were divided into three groups: a control group, a group that exercised 20 minutes daily and one that exercised 40 minutes daily.

Researchers found the average score for all children who exercised improved on the Pediatric Sleep Questionnaire.

“Existing data suggest about 2 percent of children have sleep problems, but with 37 percent of children now considered overweight, the percentage may be much higher,” said Dr. Davis, an MCG clinical health psychologist and the study’s first author.

“We believe this study is a red flag to pediatricians to ask parents about their children’s snoring,” she said. “Not sleeping well can affect behavior and the ability to function in school. We don’t know yet if it affects development.”
“The long-term consequences of sleep-disordered breathing on children are unknown,” the study authors wrote. “There may be lasting benefits of prevention or amelioration of sleep-disordered breathing as a result of protection from neural insult during childhood.”

Dr. Davis likens sleep-disordered breathing in children to the increasing incidence of lifestyle-related type 2 diabetes. “Nobody used to think type 2 diabetes happened in kids, either,” said Dr. Davis, who studies the impact of exercise on the risk of the disease in children. “We thought type 2 diabetes was something you got at maybe 50, not 15. It has become a major media sensation because it is so shocking.”

When Georgia researchers first gave the Pediatric Sleep Questionnaire to parents, they were surprised by how many children tested positive for symptoms including snoring, loud breathing and daytime inattentiveness.

Interestingly, sleepiness was not an issue; sleep-deprived children are more likely to be hyperactive than listless. Caffeine may also subvert sleepiness, the researchers said.

To learn more about sleep patterns in overweight children, Dr. Davis has started a similar study using wristbands to record movement during sleep and fingertip pulse oximeters to measure oxygen levels.

Dr. Amy R. Blanchard, pulmonologist and director of the MCG Georgia Sleep Center, is working with Dr. Davis on the new study and hopes their monitoring approach will prove an effective, inexpensive and unobtrusive way to identify early problems.

“It may give us an early diagnosis of something that could potentially affect their outcome in many ways,” said Dr. Blanchard. “The published study suggests we need to be looking more diligently at kids, not necessarily just kids with big tonsils who snore, but any child who is snoring or heavy.”

The published research was funded by the National Institute of Diabetes & Digestive & Kidney Diseases.

Toni Baker

We believe this study is a red flag to pediatricians to ask parents about their children’s snoring. Not sleeping well can affect behavior and the ability to function in school. We don’t know yet if it affects development.

–Dr. Catherine L. Davis
Unpleasantly Plump

**Chunky Teens’ Health Headed in Wrong Direction**

Key indicators of cardiovascular health, such as blood pressure and arterial stiffness, are headed in the wrong direction in “chunky” adolescents, researchers say.

“This is a wake-up call to parents and physicians to pay more attention to children who fall somewhere in the middle [of the weight continuum], because they likely are headed toward being fatter and at increased risk of cardiovascular disease,” said Dr. Yanbin Dong, an MCG geneticist and cardiologist.

He presented his findings May 10 during the Inter-American Society of Hypertension and the Consortium for Southeastern Hypertension Control Scientific Sessions in Miami.

“We tend to ignore these people,” said Dr. Dong, who looked at cardiovascular measures for 972 healthy adolescents with a mean age of nearly 18. The adolescents were part of the Georgia Cardiovascular Twin Study, led by MCG Vice President for Research Frank Treiber, on how environmental stress impacts cardiovascular health.

The 17 percent of the teens with mid-range body mass also tended to be mid-range in cardiovascular risk factors.

“Almost everything was in between,” said Dr. Dong.

For example, the systolic blood pressure of mid-range whites was about 2 mmHg higher than that of slim study participants—a risk factor for eventual hypertension, said Dr. Dong, noting, “Blood pressure in adolescence will track to adulthood, so amplification is likely as they get older.”

In blacks and whites, incremental increases in blood pressure even showed up at night, when pressures are generally lowest. Also, the heavier the adolescent, the more sodium secreted in the urine, an indicator of higher sodium intake. Excessive dietary intake may increase blood pressure, Dr. Dong said.
Arterial stiffness in the dorsalis pedis, the artery that supplies the top of the foot, also measured incrementally higher in whites based on weight. Healthy-weight blacks typically have higher arterial stiffness than whites.

Heart rate increases based on weight were fairly dramatic in blacks and whites: one beat per minute per category of slim, mid-range and heavy. And Dr. Dong noted a linear increase in the size of the pumping chamber of the heart—an indicator it’s working harder—from the thinnest to heaviest adolescents.

“Youth at risk of overweight compared with healthy-weight youth appear to have increased cardiovascular risks,” the researchers wrote. “Although there is a continuum of cardiovascular risk across all levels of [body mass index], our data suggest that the at-risk-of-overweight status already has clinical implications in youth.”

Dr. Dong’s research is funded by the National Institutes of Health and the American Heart Association. A Consortium for Southeastern Hypertension Travel Award enabled him to present at the joint research session May 6-10 in Miami.

“This is a wake-up call to parents and physicians to pay more attention to children who fall somewhere in the middle.”

—Dr. Yanbin Dong
A titanium dental implant coated with proteins that induce bone formation is a key advancement in treating tooth loss due to gum disease, researchers say.

In laboratory tests, MCG researchers applied a protein that directs stem cells into bone-forming cells onto a dental implant. The result was a nearly complete regeneration of lost tissues, said Dr. Ulf Wikesjo, MCG professor of periodontics.

Tissue loss including bone is a common and devastating result of gum disease.

Dr. Wikesjo is researching wound-healing and tissue regeneration with a $1.4 million grant from Nobel Biocare, a leading manufacturer of dental implants and equipment. Finding the key to regeneration is like putting the pieces of a puzzle together, he said.

“For the past 20 years, there has been a quest to regenerate tissues around teeth that are lost due to periodontal disease,” he said. “I’ve looked at multiple approaches to achieve regeneration, including bone grafts, root-conditioning and membrane devices for directed tissue growth, all resulting in some regeneration. We had to look at the commonalities among these treatments.”

Dr. Wikesjo and his colleagues found that regeneration requires a stable wound and space for the regenerated tissue to grow during the first stages of healing.

“If these components are in place, regeneration of the tissues around the tooth may occur within a week or two,” he said. “After that, it’s a matter of the wound maturing—going through the various stages of healing that we’re already familiar with.”

By experimenting with treatments and discerning their effects on healing bone defects, they identified some that actually hindered tissue regeneration. Some of those are considered state of the art and used in clinics today.

“Some biomaterials like hydroxyapatite particles, which are chemically similar to the mineral component of bone, may actually interfere with regeneration,” Dr. Wikesjo said. “They may not resorb quickly enough and may block the space for new tissue to grow into.”

The information helped researchers narrow possible treatments to the use of proteins that direct stem cells into bone-forming cells. The proteins were implanted around teeth and implants, but around teeth, the proteins caused gum cells to attack and destroy part of the tooth. The result with a coated implant was much better.

“There was almost complete regeneration,” Dr. Wikesjo said. “The result so far is that the implant generates its own bone that will bond with existing bone in the jaws.” The next step is clinical trials of the implant, which Dr. Wikesjo hopes to start soon.

“In some cases, the protein may rapidly release from the implant, and other times, there appears to be a more gradual release,” Dr. Wikesjo said. “We need to find out what factors cause that. In the end, we may not need to use much protein to make the implant effective. Those are things we’re looking at now.”

Jennifer Hilliard
Halting Herpes

Vaccine May Put Brakes on Common Infection

The first vaccine for genital herpes, a contagious infection affecting nearly one in five Americans, is under study in women.

MCG is among sites in 28 states studying the vaccine in approximately 7,500 women age 18 to 30 who have not been exposed to herpes simplex type 2, the cause of the genital infection, or herpes simplex type 1, which causes common fever blisters.

“It’s very debilitating, not only physically, but emotionally,” said Dr. Daron G. Ferris, director of the MCG Gynecologic Cancer Prevention Center and a principal investigator. “We hope this vaccine can help women avoid this lifelong infection.”

Previous research, published in 2002 in the New England Journal of Medicine, showed the vaccine works best in women who have not been exposed to either herpes strain and that it is not effective in men.

Antiviral agents on the market suppress outbreaks of the virus but don’t stop disease transmission. “There is no cure for herpes,” Dr. Ferris said. “People do shed herpes asymptomatically, so even if they do not have an outbreak, they can share herpes, for example, in vaginal secretions or urine.”

And the infection can be deadly for babies, who are delivered by Caesarean section if the mother is known to have an active type 2 herpes infection.

The durable virus hibernates in the dorsal ganglion, an area of nerves in the back. Stress and sunlight are two triggers that send the virus down the nerve pathways to cause an eruption.

The first outbreak is typically the worst, starting with a burning, itching sensation followed by blisters on both sides of the genital region that rupture and form ulcerations that can last up to 10 days. Recurrences generally occur on one side of the body and last about a week. Outbreak frequency varies widely among individuals. “I have patients who have an outbreak just once a year or every other year but, unfortunately, they remain contagious,” Dr. Ferris said.

The vaccine uses a fragment of the herpes virus protein that prompts the body to mount an immune response to
herpes type 2 so it will eliminate the virus on sight. It will be given in a series of three shots to approximately half the study participants. The other half get hepatitis A vaccine, which works in a similar fashion and is approved by the Food and Drug Administration to prevent infection with this hepatitis type, common in Central and South America and much of Asia. The hepatitis A vaccine also is considered investigational for the purposes of this study because it is being given in three rather than the usual two doses.

Participants will be followed for about 20 months.

The Herpevac Trial for Women is a joint initiative of the National Institute of Allergy and Infectious Diseases and GlaxoSmithKline Biologicals.

“...It’s very debilitating, not only physically, but emotionally. We hope this vaccine can help women avoid this lifelong infection.

—Dr. Daron G. Ferris

Toni Baker
A transporter that silences one of the body’s natural pain killers may yield non-addictive pain medicines and a better understanding of AIDS patients’ increased pain perception, researchers say.

Opioid peptides are natural pain relievers with receptors throughout the body, said Dr. Vadivel Ganapathy, chair of the MCG Department of Biochemistry and Molecular Biology. Studies have shown that opioid peptide levels increase, for instance, during childbirth.

Many potent pain killers, such as morphine and codeine, override this natural pain control system by directly activating opioid peptide receptors. While pain control is effective, it comes at a price: potential addiction, immune suppression and constipation.

Researchers want to know whether safer pain killers can be developed that augment the body’s natural pain-killing ability by targeting the opioid peptide transport system that terminates pain-control communication. “This has the potential for non-addictive pain killers that are effective, but by a different mechanism,” said Dr. Ganapathy.

Many popular antidepressants work in a similar fashion to keep the body’s natural chemical messengers, called neurotransmitters, working longer by keeping them from being taken back up into the neuron by transport systems. Chemical messengers are supposed to have limited action, but depressed patients have insufficient levels of chemicals for adequate communication between neurons. Antidepressants provide more mileage from existing neurotransmitters.

Dr. Ganapathy hopes to do the same with endogenous pain killers. He is using a $286,000 National Institute of Drug Abuse grant to clone the opioid peptide transporter he identified three years ago and identify the responsible gene and protein.

He also has found that mice expressing one of the HIV proteins have increased activity of this transporter, a finding that might help explain why HIV patients have increased pain perception. “That means the normal endogenous activity of this transport system is higher.”
in HIV patients, so natural pain mechanisms are not working that well,” said Dr. Ganapathy.

The National Institute on Drug Abuse asked him to apply for the Cutting Edge Basic Research Grant—enabling quick review for funding of novel ideas—to pursue this hypothesis as well as the molecular analysis of the transporter.

The idea that a transport system is involved is itself a novel concept. Conventional wisdom has held that an enzyme hydrolyzes, or decomposes, opioid peptide. This is the case for at least one neurotransmitter—acetylcholine, implicated in Alzheimer’s disease—which is inactivated by an enzyme, rather than being transported back into the neuron like other neurotransmitters.

But when Dr. Ganapathy watched the activity of opioid peptides, he saw it actively taken back up into the neuronal cells.

Cloning the transporter and dissecting its molecular profile will ultimately provide a model for studying whether drugs block this re-uptake. In fact, the National Institute on Drug Abuse will provide Dr. Ganapathy with a number of synthetic opioid peptides to see whether they are substrates, or blockers, of this transport system.

“We know this recognizes peptides. Therefore, any chemical compound that will block the transport function must have some resemblance to its natural substrate. Otherwise, it might not do the job,” Dr. Ganapathy said.

He is already testing naturally occurring amino acids and peptides and has found one—lysine, an essential amino acid vital to good growth found in red meat, cheeses, poultry, sardines, nuts, eggs and soybeans—that’s a pretty good fit.

“We may be able to take this compound as a starting point, then add a few things to make it more effective.

Dr. Vadivel Ganapathy

“We may be able to take this compound as a starting point, then add a few things to make it more effective. You could have a lysine-based drug for the treatment of pain, maybe even a nutritional supplement to prevent pain,” said Dr. Ganapathy. “You could use such a treatment to block this transport activity for HIV patients as well as other patients to control pain.”

Toni Baker
T Cell Patrol

Researchers Trace Programming of Immune System

Regulatory T cells, which function like immune system police, learn early in life what to protect, and that may include viruses, bacteria and tumors, researchers have shown.

All T cells are made in the bone marrow, then move to the thymus where they differentiate, up-regulating surface receptors, which are molecules that detect different antigens. It’s a brutal process—95 percent of the cells die in the thymus primarily because they recognize body tissue—that winds down after puberty.

Using genetically manipulated mice and technology that enables a snapshot of the antigen receptors that determine what cells recognize, MCG researchers followed T cells as they moved from the thymus to the rest of the body.

They found that regulatory T cells learn in the thymus what to protect and that some of the information may be faulty, according to research in the August 2006 issue of Immunity.

It has been widely believed that regulatory T cells recognize only endogenous body tissue so they can stop T cells that are predisposed to attacking it, said Dr. Leszek Ignatowicz, MCG immunologist and the study’s corresponding author.

But by examining receptors on all types of T cells before and after they leave the thymus, researchers found regulatory T cells can recognize endogenous tissue and invaders, Dr. Ignatowicz said.

Unfortunately, the cells may not learn to recognize all endogenous tissue, a limitation that can lead to autoimmune disease.

T cell schooling in the thymus peaks in the first six weeks of life in the mouse, which roughly translates to the first 15 years of human life. Those early lessons seem to last a lifetime, and the few regulatory cells that develop later...
will be like the early cells, said Dr. Rafał Pacholczyk, MCG immunologist and lead author.

The findings mean that essentially from the beginning, some people may have regulatory T cells less skilled at keeping the immune system from attacking their bodies and/or too skilled at protecting invaders.

The research offers hope that the cells can be manipulated to vaccinate against diseases such as lupus, arthritis and type 1 diabetes. Or more cells might be added to protect those with inadequate police.

“We need some regulatory cells more than others,” said Dr. Ignatowicz. “We probably need more of the ones that recognize autoantigens on the pancreas and [fewer of] the ones that recognize tumors.”

The fact that most regulatory T cells come directly from the thymus, not from other circulating T cells, also was previously unknown. “Where they come from is the main question we wanted to answer,” said Dr. Ignatowicz.

It has been thought that some T cells circulating in the body might make the transformation, possibly because of what they are exposed to in the body. In fact, T cells most aggressive at attacking endogenous tissue likely would be among those converting to protective regulatory cells. “We did not find that does not happen, but it’s not the major mechanism for generating regulatory cells in the body,” Dr. Pacholczyk said.

Another key question was how regulatory T cells, which make up about 5 percent of the total T cell population, can control millions of roaming T cells. They found it was a simple matter of numbers: by wearing many hats, or antigen receptors, regulatory T cells can keep their eyes on a lot of non-regulatory cells.

“The next question we will ask, which is a hot topic right now, is what antigens trigger receptors on regulatory T cells?” said Dr. Pacholczyk. “What do they recognize? We know now they are coming from the thymus, but how they are being generated is still a question. We want to look into the nature of antigens those receptors recognize, which will allow us to predict more how they are being developed in the thymus.”

Other study authors include Dr. Hanna Ignatowicz, geneticist, and Dr. Piotr Kraj, immunologist.

The research was funded by the National Institutes of Health and the Roche Foundation.

Toni Baker
A molecule expressed during pregnancy seems to make the immune system more tolerant of welcome visitors such as a fetus or transplanted organ, researchers say.

Human leukocyte antigen G, or HLA-G, is a member of a gene family called major histocompatibility complex that provokes an immune response. But like an errant child, HLA-G instead promotes tolerance, and researchers have found it can make other gene family members more accepting, said Dr. Anatolij Horuzsko, MCG reproductive immunologist. He presented his research during the Fourth International Conference on HLA-G in July 2006 in Paris. His research also was featured in the August 2006 issue of the European Journal of Immunology.

The placenta expresses HLA-G upon conception and the molecule disappears toward the end of pregnancy. Growth factors and cytokines—signaling compounds involved in the immune system—bring to the surface inhibitory receptors previously buried inside immune cells so they can interact with the HLA-G.

Amazingly, scientists have documented this natural immunosuppression in organ transplantation. Dr. Horuzsko wants to enhance this process to preclude a lifetime of generalized immune suppression for transplant recipients.

He has created animal models that express these inhibitory receptors on the cell surface. Using the same mixture the body uses—cytokines and growth factors—he also gets the receptors expressed on the surface of human cells in a test tube. He gives HLA-G in both situations and studies the response.

In dendritic cells, major orchestrators of the immune response, he has watched how activated inhibitory receptors down-regulate the function of stimulators of the immune response also present on the cell surface. Interestingly, the targets are members of the major histocompatibility complex family to which HLA-G belongs.

“These dendritic cells are not defective, but they develop tolerogenic properties, which are not normal for them,” said Dr. Horuzsko. “We think HLA-G will get them to ignore the antigen coming from transplanted tissue.” Unfortunately, he noted, cancer and some viruses seem capable of similar manipulation.

Using this approach, he has been able to prolong acceptance of skin grafts, which are typically rapidly rejected. He also is prompting T cells, another major player in the immune response, to express inhibitory receptors.

Normally, dendritic cells prompt T cells to destroy invaders. But when T cells express the inhibitory receptor, they go silent and may even die.

A long list of other cells that provoke an immune response might be controlled by this approach, Dr. Horuzsko said.

He envisions giving cytokines and growth factors to patients so targeted cells will express inhibitory receptors, then delivering blood-derived stem cells modified to produce HLA-G. HLA-G alone might be sufficient for patients who already express inhibitory receptors.

Toni Baker

Welcome Mat

Pregnancy Molecule May Aid Transplant Recipients

Doctor Anatolij Horuzsko
CORRECTION
An article in the March 3, 2000 edition of Science that was referenced in the 2001 MCG Tomorrow article, “Clues Found in Process of Memory,” was retracted Dec. 15, 2006 by the authors. The authors have found that, contrary to the information published in the article, Calcyon and the dopamine receptor, D1, do not directly interact. The scientists regret that errors leading to the conclusions about the interaction were not found before publication.
Two genes important for human development and implicated in cancer and schizophrenia also help keep a healthy balance between excitation and inhibition of brain cells, researchers say. Neuregulin-1 and its receptor, ErbB4, promote inhibition at the site of inhibitory synapses in the brain by increasing release of GABA, a major inhibitory neurotransmitter, researchers reported in the May 24, 2007 issue of Neuron.

In 2000, a research team, also led by Dr. Lin Mei, showed that neuregulin-1 and ErbB4 also are at excitatory synapses, communication points between neurons where the neurotransmitter glutamate excites cells to action. Here, neuregulin-1 and ErbB4 suppress excitation.

"Right beside the place where the excitatory synapse can be activated, there is also something that can suppress it," said Dr. Mei, MCG chief of developmental neurobiology. "Now, we have identified another novel target of neuregulin-1 which is the inhibitory synapse."

Together, the findings reveal a check and balance for brain cell activity managed by neuregulin-1 in the brain’s prefrontal cortex, where complex reasoning and decisions about appropriate social behavior occur. They also provide new treatment targets for diseases such as schizophrenia and epilepsy.

The genes are both associated with schizophrenia, a disease that affects about 1 percent of the population, but the exact role of malfunctioning neuregulin-1 signaling was unclear.

"[Dr. Mei’s] findings help explain how a gene that is potentially causative in disorders like schizophrenia and bipolar disorder relate to a neurotransmitter that is critical for explaining the cognitive deficits associated with the illness," said Dr. Daniel R. Weinberger, director of the Genes, Cognition and Psychosis Program at the National Institute of Mental Health in Bethesda, Md.

"What we have found is neuregulin-1 can regulate GABA release from these neurons, and if the GABA is released here, that may play a role in controlling the output of this neuron," Dr. Mei said, pointing to an illustration of pyramid-shaped neurons that looks like a high-tech switchboard with information coming in from all angles.

Pyramidal neurons get information from nearby interneurons, integrate it, then decide what message to move forward. “This pyramidal neuron receives inhibitory input and excitatory input, and neuregulin-1 can regulate both,” said Dr. Mei.

They nicely balance input in healthy brains, enabling people to balance their checking accounts and suppress the urge to run naked down the street.

In 2006, University of Pennsylvania researchers reported in Nature Medicine an altered signaling pathway for neuregulin-1 and ErbB4 genes in the brains of schizophrenics. Dr. Mei’s findings show that these factors associated with a schizophrenic brain have at least two places to act.

"There is a ton of evidence that when inhibitory synapses, such as GABA, go wrong, the symptoms of mice and rats look similar to those of schizophrenia in people," he said.

Mounting evidence suggests that problems with the excitatory and inhibitory synapses regulated by neuregulin-1 result in other problems as well: Excess excitation results in seizures and excess inhibition in depression, as examples.

"If this neuron is too excited, people may get manic or have seizures," said Dr. Mei. “Patients with schizophrenia, for example, show symptoms that implicate alterations in inhibitory
neurotransmission in addition to excitatory neurotransmission.”

Dr. Mei co-authored a companion paper in *Neuron* with scientists at Cold Spring Harbor in New York that provides yet another link between neuregulin-1, its receptor ErbB4 and schizophrenia. It shows ErbB4 plays a key role in the maturation and plasticity of excitatory synapses and that normal synapse development is impaired by genetic defects in neuregulin-1 and ErbB4 signaling. The result is impaired function of the excitatory neurotransmitter, glutamate.

Now, he wants to study disease processes in a neuregulin-1/ErbB4 knockout mouse and learn more about how neuregulin-1 mediates GABA release. Another key unknown is what regulates neuregulin-1.

*Toni Baker*
**Special Delivery**

**Motor Protein Vital in Connecting Neurons**

A motor protein called myosin X runs the main road of a developing neuron, delivering to its tip a receptor that enables it to communicate with other neurons, scientists say.

In another piece of the puzzle of how neurons form connections, researchers have found myosin X travels a portion of a neuron’s backbone called the actin filament, a sort of highway in the cell’s highest traffic area, said Dr. Wen-Cheng Xiong, an MCG developmental neurobiologist.

Part of its cargo is DCC receptor, which needs to move from the central nucleus where it is synthesized to the cell’s periphery, Dr. Xiong and her colleagues reported in the February 2007 issue of Nature Cell Biology. The paper was recently featured in the Editor’s Choice section of Science Magazine’s Signal Transduction Knowledge Environment site (www.stke.org) as a noteworthy contribution to scientific literature.

At the periphery, DCC interacts with netrin-1, a guidance cue for helping the arm-like extension of the cell, called the axon, grow in the right direction. Cells eventually communicate through synapses at the end of these cellular projections.

“During early development, axons need to find a target, decide how long to grow and in which direction to grow. Eventually, they will form a synapse,” said Dr. Xiong, who hopes a better understanding of the process will help restore communication in spinal cord patients.

“Growth is precisely controlled during development,” she said, ensuring proper brain wiring and connectivity. “Myosin X gets the DCC receptor where it needs to be so it can interact with netrin-1.”

Her previous studies, published in 2004 in *Nature Neuroscience*, showed that DCC binding to netrin-1 activates an enzyme, focal adhesion kinase, enabling developing cells to reorganize and know how to move. The process enables brain cells to reach out to each other and across the midline of the developing brain and spinal cord. When the kinase is deleted, the axon doesn’t make the proper connections.

When researchers cut off myosin X’s motor—which they believe happens in spinal cord injuries—axon outgrowth also was hindered.

“Myosin X plays a critical role in neurons during development,” said Dr. Xiong. Different versions of the myosin family proteins are critical to essentially every cell.

The rapidly moving protein is easily degraded and needs tight regulation. “If you don’t want to have dramatic changes in your neuron structure, you don’t want this molecule,” Dr. Xiong said.

In fact, she suspects the function of myosin X changes as the neuron develops. She has documented that late in development, when the axon should stop growing, a shorter molecule, minus the motor, is expressed.
Growth is precisely controlled during development. Myosin X gets the DCC receptor where it needs to be so it can interact with netrin-1.

–Dr. Wen-Cheng Xiong

“Probably after the neuron is developed, the major work of myosin is done. There are many cleavage sites in the middle, and this typically large molecule can be cut down to a small molecule that actually inhibits axon growth function,” Dr. Xiong said.

She suspects that negative function surfaces when the spinal cord is cut and plans to examine whether the protein is degraded in spinal cord injuries. “We already have evidence that if this protein degrades, most frequently without its motor domain, it becomes negative, inhibits DCC getting to the proper place and so axonal growth,” Dr. Xiong said.

The work was funded by the National Institutes of Health. Co-authors include Dr. Xiao-Juan Zhu, former postdoctoral fellow in Dr. Xiong’s lab, Dr. PengGao Dai, former postdoctoral fellow in Dr. Lin Mei’s laboratory at MCG and Dr. Yu-Qiang Ding’s laboratory at the Institute of Neuroscience and Key Laboratory of Neurobiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Toni Baker
Recovery in Motion

*Stem Cell Transplants Improve Recovery in Animal Models for Stroke, Cerebral Palsy*

A single dose of adult donor stem cells given to animals with neurological damage can significantly enhance recovery, researchers say.

Using a commonly utilized animal model for stroke, researchers administered 200,000 to 400,000 human stem cells into the brains of animals with significant loss of mobility and other functions. The stem cells used in the study were a recently discovered stem cell type called multipotent adult progenitor cells, or MAPCs.

Motor and neurological performance in the animals improved 25 percent, said Dr. Cesario V. Borlongan, a neuroscientist at MCG and Augusta’s Veterans Affairs Medical Center.

The findings were presented in April 2006 during the 58th annual meeting of the American Academy of Neurology in San Diego.

In humans, the findings ideally will translate into incremental but important recovery advances, said Dr. David Hess, chair of the MCG Department of Neurology and a study co-author.

“The single largest cause of disability among adults in the U.S. is stroke,” said Dr. Hess. “It’s a huge public health problem in the world.” He hopes stem cell therapy, aggressive physical therapy and the clot-dissolving drug, tPA, can work synergistically to reduce that disability.

“These are not going to be cures, but this level of recovery is significant. If somebody can go from a wheelchair to a cane, that is a big improvement,” Dr. Hess said.

Adult animals were tested across a range of standardized tasks both before and after a surgically induced stroke.
Following the stroke, both control animals and those that received a single injection of stem cells were evaluated for up to two months. Animals treated with stem cells had improved strength, balance, agility, fine motor skills and tissue recovery.

“A single dose of the cells produce robust behavioral recovery at an early period post-transplantation, and the recovery was durable, lasting up to two months, which was the entire length of this study,” Dr. Borlongan said. “Furthermore, animals continued to show improvement over time.” In the newborn model of ischemic injury, enhanced recovery took place within two weeks.

Even though fewer than 1 percent of the transplanted cells were present two months later, animals receiving treatment developed new neurons, apparently formed from endogenous stem cells. “The mechanism that we are putting forward is these donor cells are secreting nourishing trophic factors that are helping the host brain cells survive and stimulating stem cells from the host to multiply,” Dr. Borlongan said.

To help mimic potential clinical scenarios for stroke victims, transplants were performed seven days after the initial injury. The drug, tPA, must be administered within three hours of a stroke, a window most patients miss.

In the adult stroke model, MCG researchers found stem cell treatment increased the number of injured cells that survived just outside the area of greatest damage, also referred to as the ischemic core, by up to 20 percent.

“Up to this point, all the treatment approaches, including transplantation and tPA, cannot get rid of that ischemic core,” Dr. Borlongan said. “But outside of that core is a lining, the penumbra, which if you do not treat over time becomes part of the core. We are showing that even one week after a stroke, we are able to increase the number of cells surviving along that penumbra, and that is how we feel it is producing significant recovery.”

Animals in a model of cerebral palsy, a condition caused by an ischemic injury before or during birth, also improved at least 25 percent more than controls. Rodent stem cells were used in this model. A larger percentage of donated cells survived and within two weeks matured into neurons in the young, more pliable brains, Dr. Borlongan said. Also, close donor matching seemed unnecessary. Unmatched transplants from the same species and genetically identical transplants yielded comparable results.

Athersys, Inc., a Cleveland-based biopharmaceutical company, funded the research in which previously frozen human or rodent multipotent adult progenitor cells (MultiStem™) were thawed and injected directly into the brain.

Researchers believe MultiStem™ cells help in multiple ways, for example by producing factors that limit tissue damage and stimulate repair, according to Dr. Gil Van Bokkelen, the company’s chair and chief executive officer. The cells also can safely mature into a broad range of cell types and can be produced on a large scale.

In extensive animal testing, the mature stem cells have been shown to be safe and generally do not require close genetic matching, according to company executives. Another advantage is immunosuppressive drugs do not appear to be required, as they are with other types of stem cell treatment.

Although they have not specifically looked at whether stem cell therapy might be useful months after an ischemic event, the researchers believe early therapy likely will be the most successful.

They already are working with the Food and Drug Administration to begin clinical trials within the next few years.

In preparation for potential clinical use, the researchers are pursuing transplants in larger animal models and studying how MultiStem™ cells work in living human brain tissue housed at the MCG Human Brain Bank.

Toni Baker
Transplanted neural stem cells hold promise for Parkinson’s disease, scientists say.

Research published in the Nov. 29, 2006 issue of *The Journal of Neuroscience* shows a human neural stem cell transplant enables an animal with Parkinson’s to continue functioning normally rather than displaying the progressive loss of movement control that characterizes the disease.

“We are very cautious, but to us, it’s an indication that stem cells have promise for Parkinson’s disease,” said Dr. Cesario V. Borlongan, an MCG neuroscientist and corresponding author of the study.

The rats in the study received the transplanted cells shortly after Parkinson’s was induced by destroying neurons that make dopamine, a neurotransmitter key to movement control. This would be equivalent to a patient getting treatment very soon in the disease process, which rarely happens since there is no screening test to catch it early.

“If you are able to identify Parkinson’s in the early stage, we think this therapy will work,” said Dr. Borlongan. “An important question that remains is, ‘Can we rescue neurons that are dying from Parkinson’s?’ This would more accurately mimic what patients need.”

The researchers already have begun studies that delay the transplants until weeks after injury.

For this study, researchers compared animals that received placebo treatment with those that received only protective neurotrophic factors secreted by stem cells and those that had a transplant.

Animals that received transplants regained movement control, placebo-treated animals did not recover and those that received neurotrophic factors, called stem cell factors, recovered partially.

When researchers examined the brains one month after transplant—a long time in the two-year life of a rat—researchers found endogenous dopaminergic cells and transplanted neural stem cells had both survived. Also, transplanted neural cells had formed synapses to communicate with each other and ultimately the striatum, the portion of the brain dopaminergic cells act on to control movement.

“The transplanted stem cells survived, differentiated into neurons and showed some connection with the host tissue,” said Dr. Borlongan.

They did additional studies in test tubes, taking commercially available rat and human dopaminergic cells, exposing them to neurotoxins and then to stem cell factors. “The more stem cell factor, the better the protection,” Dr. Borlongan said. When the cells were co-cultured with stem cells, protection was further increased. When they used an antibody to block the stem cell factor, neuro-protection was significantly reduced.

“This again shows a combination of factors at work,” said Dr. Borlongan. “It’s a synergistic effect.”

The standard treatment for Parkinson’s disease, which affects about half a million Americans, is L-dopa, a synthetic dopamine that typically minimizes symptoms for three to five years. As the disease progresses and the drug becomes less effective, doses are increased and can produce more dyskinesia, or loss of controlled movement. Centers such as MCG’s are exploring new ways to slow disease progression, diagnose it earlier and more accurately monitor its progression.

Toni Baker
Dr. Borlongan’s research was supported in part by Veterans Affairs Merit Review funds.

Stem cells were provided by Dr. Seung U. Kim, Professor Emeritus of neurology at the University of British Columbia in Vancouver and a study co-author. Other co-authors include Drs. Koichi Hara, Mina Maki, Noriyuki Matsukawa, Takao Yasuhara and Guolong Yu, MCG postdoctoral fellows, and Dr. Lin Xu, an MCG researcher.
Adults with a genetic variation enabling them to express higher levels of fetal hemoglobin may have a reduced risk of Alzheimer’s disease, researchers say.

A study of 209 families with at least two siblings with Alzheimer’s and one unaffected sibling showed that those with this genetic variation are less likely to have the disease, researchers said in Neurobiology of Aging. An estimated 25 percent of the population has the Xmnl polymorphism.

The study also showed that beta amyloid peptide, a major culprit in Alzheimer’s, has an affinity for adult hemoglobin, said Dr. William D. Hill, a neuroscientist at MCG and Augusta’s Veterans Affairs Medical Center and a corresponding author.

The hemoglobin attraction was discovered by using phage display technology to screen thousands of molecules in the human brain to find those that interact with beta amyloid peptide. This approach uses a virus to infect a bacterium so the bacterium will copy the virus. The result looks like a microscopic cigar, with the proteins of interest as whisks on one end. In this case, a library of brain molecules was inserted into the virus’ whisks to find proteins that would stick to beta amyloid.

Hemoglobin, a component of red blood cells that carries oxygen in the body, was among those that stuck.

Surprised that hemoglobin was even present, Dr. Hill suspected it was an artifact of preparing brain tissue for the library. But once he saw the attraction, he could not ignore it.

His lab actually first found an attraction for fetal hemoglobin, another surprise since most adults have little of this substance that snatches oxygen from the placenta and holds onto it tightly for the fetus. Looking further, his lab found adult hemoglobin was binding as well, so Dr. Hill and Drs. Abdullah and Ferdane Kutlar decided to study the Xmnl polymorphism, which can significantly increase fetal hemoglobin expression in adults. They turned to colleagues at the University of Alabama at Birmingham, one of three sites that contributed family data to the National Institute of Mental Health Alzheimer’s databank.

At the UAB databank, headed by Dr. Rodney C.P. Go, researchers found more surprises. “We wanted to look at
people who had Alzheimer’s and family members who don’t to see who expressed the polymorphism the most,” said Dr. Hill. They suspected it would be the Alzheimer’s patients and found just the opposite.

In what they suspect to be a vicious cycle, beta amyloid could injure red blood cells, allowing more of them than usual to break open and spill their contents, including oxygen-carrying hemoglobin, into the bloodstream. Free hemoglobin is toxic; it can easily lose its iron group, causing cell-damaging oxidative stress. Now, it appears freed hemoglobin may also bind to beta amyloid, which may enhance that protein’s ability to wreak havoc in the brain.

Red blood cells break down daily, and molecules that bind free hemoglobin and iron take them to the liver for elimination. “Part of our hypothesis is that it may be free-radical injury of our red blood cells by the beta amyloid that releases excessive hemoglobin, which overwhelms our body’s natural system for protecting us from free hemoglobin,” Dr. Hill said.

Work in the late 1990s showed fragments of beta amyloid can attack red blood cell membranes and cause more fragility in some Alzheimer’s patients, “There is some evidence that red blood cells of Alzheimer’s patients have been damaged, so we think red blood cells are more fragile in some Alzheimer’s patients.” Dr. Hill said.

More Alzheimer’s patients and their families must be studied to determine the impact of the genetic mutation on Alzheimer’s risk, according to Dr. Rodney T. Perry, a UAB molecular geneticist and a study corresponding author who is submitting a grant with Dr. Hill to pursue more answers. Their theory of fetal hemoglobin’s protective role will be strengthened if higher levels are found in healthy family members with the XmnI polymorphism.

The findings could open the doors to treatments that increase fetal hemoglobin levels. One such drug, hydroxyurea, is used to treat sickle cells patients because fetal hemoglobin will not sickle.

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—Dr. William D. Hill

Toni Baker

The researchers found that in certain circumstances, adult hemoglobin binds better to beta amyloid, so higher levels of fetal hemoglobin may protect against injury.
Energy Booster

**Creatine May Be Shot in the Arm for Dying Cells**

A North American study is investigating whether a supplement used by athletes to boost energy and build muscle can slow progression of Parkinson’s disease.

Creatine, under study for neurological and neuromuscular diseases such as Lou Gehrig’s and muscular dystrophy, may give an energy boost to the dying cells of Parkinson’s patients, said Dr. Kapil D. Sethi, neurologist and director of the MCG Movement Disorders Program.

“We think it may help cells that are damaged or overworked,” said Dr. Sethi, a site principal investigator on the National Institute of Neurological Disorders and Stroke study. MCG hopes to recruit 45 patients for the study that will enroll 1,720 patients at 51 sites in the United States and Canada.

Mitochondria, the powerhouse for cells, become dysfunctional in the brain, muscle and platelet cells of many patients with Parkinson’s disease. “By giving more energy to the cells, you are giving them a safety margin,” Dr. Sethi said. “If a cell is dying, it takes another route [to survive].”

The goal is to slow progression of the disease that affects about 1 million people in North America. Symptoms include tremors, rigidity, slowed movement and, late in the disease, dementia and behavior disorders.

Treatments—including the gold standard, a synthetic dopamine called levodopa and MAO-B inhibitors that forestall breakdown of dopamine—target those symptoms. Dopamine, a neurotransmitter critical to movement, is depleted in Parkinson’s. Researchers hope newer therapies, including creatine, can be added to the mix to help slow the disease.

The study will enroll patients who have been on standard therapies from 90 days to two years and follow them for five years. Half the enrollees will get creatine and half placebo. Researchers hope for at least a 20 percent reduction in disease progression so that in five years, patients on creatine will look like placebo patients at four years, said Buff Dill, MCG study coordinator.

The disease is assessed by following its progression long term and measuring endpoints such as falls, nursing home placement, dementia and death. The study may be extended five years, based on preliminary results and funding.

Those treated with creatine may also build muscle—a significant benefit since Parkinson’s patients often experience muscle atrophy and weight loss.

Creatine is available over the counter, but “patients realize that we don’t know if it works [for Parkinson’s],” Dr. Sethi said. “They are willing to take the risk of being on placebo for the cause of science and to learn more about the disease. They want to beat this disease and if they can’t, they want to help somebody else beat it.”

Avicena Group, Inc., will provide creatine and placebo for this first large study in a series of National Institutes of Health-sponsored exploratory trials in Parkinson’s.

MCG will soon participate in a similar study of coenzyme Q10, another natural supplement that boosts energy production. Dr. Sethi, project director of the Parkinson Research Alliance of India, which is working to bring more clinical trials to his homeland, plans to incorporate these supplements into innovative treatment cocktails that will be studied there.

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