medicine at MICHIGAN
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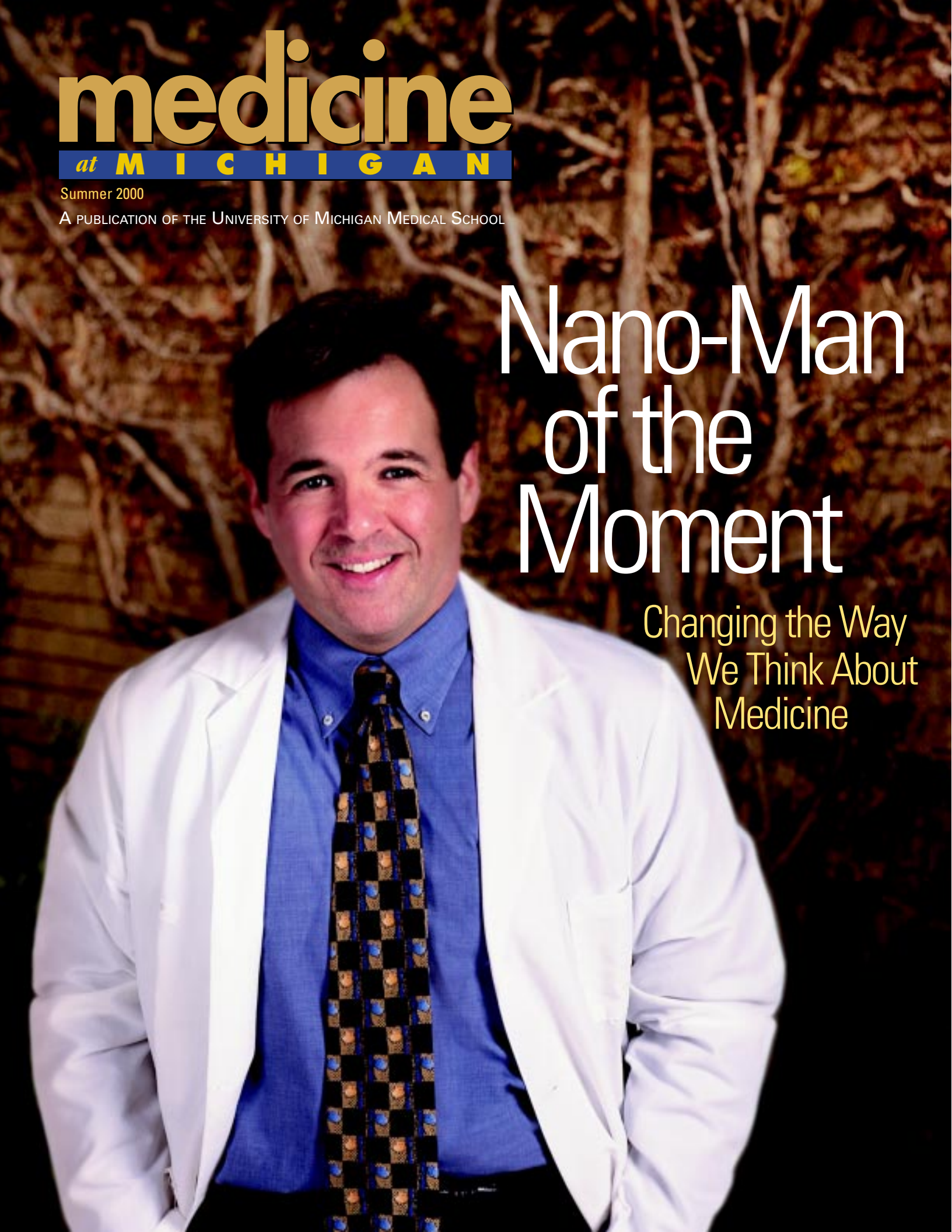


Nano-Man of the Moment

Changing the Way We Think About Medicine

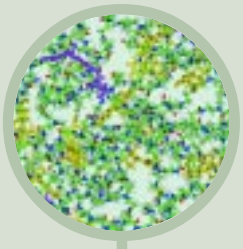
"NANO" IS "NOW" AT MICHIGAN—

AND JAMES BAKER IS LEADING THE WAY

by Eric J. Lerner

James Baker and His Colleagues at the Center for Biologic Nanotechnology Are Using Nanoengineering to Fight Infections, Deliver Genes, Destroy Cancer Cells and Completely Alter the Way We Define Medicine.

Biology Meets High Tech: Forget Leeuwenhoek. Forget those glass lenses that first brought you the magical sight of an amoeba swimming in pond water. Forget the electron microscope. Shrink your notions of small and travel into the world inspired by the microchip, a world smaller than your teeniest notion of small...a "nano" world where some of the biggest dreams ever dreamed in medicine are being dreamed today.



Imagine tiny plastic balls a zillion times smaller than anything you can think of. Imagine drops of ordinary soy bean oil a zillion times smaller than anything you can think of. Imagine the tinier-than-tiny plastic balls and the super-small drops of soybean oil saving your life.

Welcome to the fantastic new world of nanomedicine — a world where doctors, biological scientists, chemical engineers and microchip builders are all speaking the same language, working together in a new and very tiny world of truly cross-disciplinary miracles.

"This is the start of the post-genomics therapeutic revolution," says James R. Baker Jr., M.D., an allergist and immunologist by training and now director of the new Center for Biologic Nanotechnology he founded at the University of Michigan Medical School in 1998. One of the early believers in nanomedicine, Baker is a convincing proponent of this nascent field. "What we are doing is developing synthetic materials — not biologic molecules — to form tiny structures, or nanodevices, that perform medically important tasks," he explains. The Center has pioneered research in nanodevices that perform these tiny miracles. These devices are so small that they can slip inside cells without being recognized, but at the same time alter the function of entire organs "where we specifically address problems identified by genetic analysis using tailored therapies that restore normal molecular function." A number of extremely promising avenues of research are underway in the Center, including several that are advancing into clinical trials.

The whole concept of nanomaterials is difficult for the layperson to understand. By definition, nanomaterials are thousands to millions of times smaller than the cells that make up our bodies, and the ability to make materials this small is a recent accomplishment. (The prefix "nano" actually means "billionth.") Much of the basic research in these materials came from the semiconductor industry, where the need for smaller circuits and storage devices drove the miniaturization of engineered parts. These studies have led to the assembly of materials, literally atom by atom, into devices like motors and disk drives. Many of the materials Baker works with actually self-assemble into more complex arrays and devices. However, as difficult as it might be to produce these materials, it is even harder to visualize them and assure that the structure is correct. This has spawned a new industry to develop machines that can analyze and image nanodevices. "These materials are so small they cannot be viewed with traditional tools, even electron microscopes," Baker explains. "We often have to examine them indirectly, as if we are observing a shadow. It makes the whole process very difficult."

Despite the complexity of the manufacture and analysis of nanostructures, the potential benefits are truly remarkable. "We believe that most human afflictions can be addressed by nanotherapeutics," Baker says with the confidence of the laboratory-rooted dreamer that he is, and he goes on to illustrate his point. "For example, we have developed non-toxic nanoemulsions that penetrate and kill infectious microbes from the flu virus to anthrax spores. We are using synthetic molecules called dendrimers as machines to detect and characterize cancer cells, then destroy them by selecting and delivering a specific drug or gene therapy. Other work is focused on developing polymers that replace defective cellular proteins to treat genetic disorders, such as cystic fibrosis. The possibilities are truly limitless." Not that it will be easy. Scientists in nanomedicine are faced with the challenge of not only developing materials, but also adapting and inventing new tools. They have to bring a totally fresh mindset to the treatment of disease. At Michigan they're doing it with a uniquely integrated multidisciplinary approach, with ongoing collaboration between scientists from materials science, chemistry, pharmacology and medicine. "It is really more of a think tank than a traditional medical research program," says Baker with a pride he finds hard to conceal. ➤

DENDRIMERS CAN CARRY GENES INTO CELLS
THE BODY'S IMMUNE RESPONSE. "IN EFFECT, THEY

Fighting flu with vegetable oil

The project that has moved the furthest towards practical application – using nanoemulsions of vegetable oil to kill microbes — is perhaps the most dramatic demonstration of the power of nanotechnolgy. Soybean oil in its standard form does not kill any microbes — in fact quite a few use it for food. But Baker and colleagues Tarek Hamouda, Andrzej Myc, Peter Cao, Amy Shih and Brian Donovan found that if the oil is emulsified with detergents to form nanodrops 400-600 nanometers (nm) across, they act with devastating effect on nearly all pathogens. It is not a chemical action, but a physical one similar to what causes the oil and water in salad dressing to separate. The droplets' surface tension makes them want to coalesce with other lipid droplets, even though they are stabilized so they cannot coalesce with themselves. The smaller the droplets, the greater the surface tension and the stronger the urge to merge. When the oil droplets contact the membranes of bacteria or enveloped viruses, the surface tension forces a merger with the membrane, blowing it apart and killing the pathogen. "Basically what we have created are nanometer-sized bombs," says Baker. "But the tissue structure of the cells of humans and other higher organisms prevents them from being disrupted by the droplets." As a result, the emulsion is entirely safe when applied externally. Only red blood cells and sperm cells lack tissue support structure and are vulnerable to fusion and destruction by the oil droplets. This means on the one hand that the emulsion cannot be used intravenously, but it also makes it a potential anti-microbial contraceptive that kills both sperm and sexually transmitted disease organisms like HIV.

Tests in mice have been promising. When mice were given nose drops of the oil nanoemulsions and then exposed to a lethal dose of influenza virus, 75 percent were protected against the disease, while 80 percent of the control mice died. "We think the results in humans will be much better," explains Hamouda, "because the virus dosages in the experimental animals were far higher than people would be normally exposed to."

In other experiments, mice were given wounds and then infected with anthrax-like spores. Without treatment, the mice developed large infected sores, similar to cutaneous anthrax. However, mice whose wounds were washed with dilute nanoemulsion solution hours after infection developed essentially no lesion at all.

It was particularly surprising in the nanoemulsion research that bacteria spores, as well as active bacteria, were killed. Spores are especially hard to kill because they have a hard exterior coating rather than a membrane. "The oil emulsion seems to trick the spores, because it looks like food to them," says Baker. "Once the spores become active and generate a membrane, the emulsion kills them." This action has been of particular interest to the Defense Advanced Research Projects Agency that has funded some of this work, as it is a promising way to fight the use of anthrax spores in biological warfare.

Molecular toolboxes

While the nanoemulsion works well as tiny bombs, for most uses more subtle interventions are needed, using a variety of molecular tools. To deliver these tools, the Center's researchers have been developing a kind of all-purpose molecular toolbox called a dendrimer. Dendrimers are spherical, branching molecules whose structure looks like a very regular bush. They can be made in various diameters, depending on how many shells or branchings they have, and are covered with dozens of molecular "handles"(simple amino groups) that researchers can use to attach a variety of biochemically active molecules. The more shells, the larger number of amino groups — two shells have eight groups, four shells have 16, and so on. Dendrimers can act as nano-sized tool kits, able to deliver the modular tools to the right place, and even as nanomachines, able to use the tools in the right sequence at the right time.

Dendrimers were invented in the late 1970s by Donald Tomalia, now scientific director of the Biologic Nanotechnology Center. "I was looking for a way to make branching molecular structures that imitated the branching of trees," Tomalia recalls. Initially the molecules were applied for non-biological uses, but in the mid-1990s Tomalia and Baker started to look at possible medical applications, especially for gene therapy. "The great advantage of dendrimers is that they don't trigger the immune system," says Lars Piehler, a biochemist at the Center. The amino groups that cover the molecule are not recognized as foreign by the immune system, unlike the proteins of other vectors such as adenovirus or adeno-associated virus (AAV).

Perhaps the most far-reaching potential application of dendrimers in medicine is for gene and drug delivery. Despite its immense promise, gene therapy has been stymied because of the problem of getting genes into enough cells to make a therapeutic difference. Carrying the gene into the cells as part of a virus, one widely used approach, has the serious disadvantage that the virus, even if benign, triggers an immune

S WITHOUT SETTING OFF
ARE STEALTH PROTEINS."
— DONALD TOMALIA

reaction by the body. If fewer virus particles are used to minimize the immune reaction, not enough cells are altered. Unfortunately, the larger virus dosages required for most applications risk an overwhelming immune response. This can have serious consequences, including fatal reactions, as happened with the highly publicized case of Jesse Gelsinger, a teenager who died following adenovirus gene therapy.

Dendrimers can carry genes into cells without setting off the body's immune response. "In effect, they are stealth proteins," says Tomalia. Although they are big enough to carry genetic material, as a virus would, their bushy surfaces lack the complex folds that allow antibodies to bind and alert the body's immune defense. Instead, they appear as blobs of amino acids. This allows them to carry large amounts of genetic material into the body without undue stress. In addition, the size of a dendrimer, which is adjustable depending on how many shells they have, is in the right range to wrap genetic material around them.

So far, the dendrimer-based delivery or transfection of genes has been demonstrated successfully in vitro and is now being tested intensively in animals. "We've found that the efficiency of transfer — the percentage of cells that get working genes — is almost as high as with viruses," says Baker. But substantial challenges remain. Dendrimers may interfere with genetic transcription, which could prevent the transferred genetic material from functioning within cells. In addition, despite the fact that dendrimer-carried genes did not produce pneumonia when inhaled into animals' lungs, some of the animals died. This appeared to be the result of the dendrimers pulling fluid into the lungs of the animals. "All new materials will have some toxicity, often unexpected, and require extensive toxicity testing. The important thing is not to take anything for granted," remarks Baker. So several hurdles will have to be overcome to reach the goal of a general-purpose gene-delivery system using dendrimers.

A cancer-fighting nanomachine

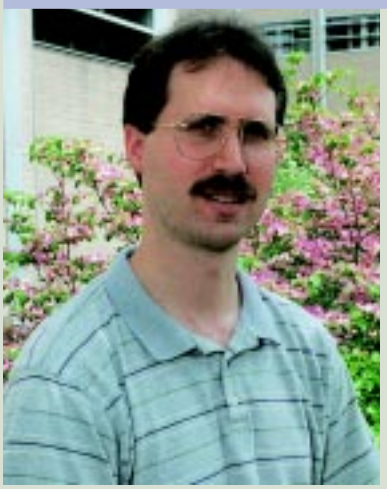
The most ambitious avenue of research in the Center is to adapt the dendrimers' toolbox capabilities to produce a multi-functional anti-cancer nanodevice. In cancer therapy, the long-sought goal has been a treatment that attacks only the cancer cells and not the healthy cells. Piehler and colleagues are working on ways to do just that — with a dendrimer that locates cancer cells, shows where they are, enters them, confirms they are cancerous, and then kills them.

"Because the dendrimer has so many attachment sites, we can put different chemicals on different sites for a variety of functions," Piehler points out. In the approach now under development, targeting groups on the ➤

Amy Shih

Research Assistant Amy Shih develops and manufactures anti-microbial nanoemulsions and tests their activity against spores and vegetative bacteria.

Lars Piehler



Lars Piehler, Ph.D., research associate, makes surface-modified dendrimers and biomolecule-dendrimer bioconjugates in order to tailor their biological activity and chemical properties.

Tarek Hamouda

Tarek Hamouda, Ph.D., research associate, oversees the development of new anti-microbial nanoemulsions with a broad spectrum of activity against bacteria, bacterial spores, enveloped viruses and fungi. These nanoemulsions will be used against biological warfare agents or for decontamination of food to avoid food-borne pathogens.

"ONE OF THE GREAT ADVANTAGES OF DENDRIMERS IS THAT YOU CAN BIND CHEMICALS AT PRECISE LOCATIONS, INCLUDING IN THE INTERIOR OF THE MOLECULE, BECAUSE OF THE WAY THEY ARE BUILT UP FROM THE INSIDE OUT."

—DONALD TOMALIA

dendrimer would attach preferentially to cancer cells rather than healthy ones. A second attached group would be fluorescent, so that the cancer cells can be viewed by surgeons using a weak laser light, or labeled for use with MRI imaging techniques. Once inside the cells, another dendrimer will confirm the "signature" of cancer within the cell, to assure normal cells have not been targeted. Inside another part of the polymer structure, the dendrimers will carry a cytotoxin, such as cisplatin. The poison will be bound up with the dendrimer and be inert until release. Then, release of the poison, which can be triggered by several methods including laser light, kills the cancer cells and gives a readout that the tumor has been killed. In this plan, laser light could be introduced to an internal tumor through a narrow fiber, minimizing surgical damage to healthy tissue.

"One of the great advantages of dendrimers is that you can bind chemicals at precise locations, including in the interior of the molecule, because of the way they are built up from the inside out," explains Tomalia. Each time a new shell is added to the dendrimer, new functional groups can be attached and then incorporated within the molecule as an additional shell is added. With multiple attachment points, several copies of each functional chemical can be used, increasing the effectiveness of the group.

Tomalia, Piehler and others are pursuing a number of possible strategies for anti-cancer nanomachines. In another version the dendrimers carry boron clusters into the cancer cells. Boron is a heavy absorber of neutrons, so when the tumor is irradiated with a neutron beam, the boron nuclei absorb energy, releasing it as short-range, but deadly, X-rays, killing the cells they are in. But the neutrons pass relatively harmlessly through the healthy cells, producing little damage.

Wrapping up a virus

The Center is also investigating the possibility that dendrimers might be used in novel ways to fight viral infections. A first step in many viral infections occurs when a virus attaches itself to the sialic acid molecules found in the human cell membrane. (Sialic acid is a sugar-coated lipid known as a glycolipid that is a component of human cell membranes.)

By coating dendrimers with low concentrations of sialic acid, it is reasoned, one could "fool" the virus into attaching itself to the dendrimers instead of to the cell itself. "We can grow dendrimers to be long and rod-like instead of spherical," explains Tomalia. "If we cover them with sialic acid, they can wrap themselves around the virus in such as way as to prevent the virus from binding to the cell." Tomalia and his collaborators have found in laboratory experiments that these multiple-branched, linear dendrimers, which vaguely resemble ivy vines, have been most effective in preventing various strains of flu viruses from binding to cells.

While much work remains to be done in this area, Tomalia and his colleagues at the Center share the heightened enthusiasm that comes from exploring a true frontier, both medical and scientific. They feel confident that the incredibly small world of nanotechnology is opening up a giant new world of medicine, one with possibilities never even dreamed of before now.

Baker, the nano-man of the moment, finds inspiration in a quote from Sir Arthur Conan Doyle: "I think that little things are infinitely more interesting."

Andrzej Myc

Brian Donovan

Peter (Zhengyi) Cao

Andrzej Myc, Ph.D. (far left), research investigator, works on neutralization of bacterial toxins using nanoemulsions and dendrimers. Research Assistants Brian Donovan (center) and Peter (Zhengyi) Cao, M.D. (right), develop cellular and mouse models to test the safety and efficacy of nanoemulsions against various

ING SMALL ON A GRAND SCALE:

From AIDS to Desert Storm, Jim Baker's Life in Medicine Has Not Been an Indifferent One. Perhaps That's Why Nanomedicine, with Its Bold New Death-Defying Promises, Holds So Much Fascination for Him.

by Jane Myers

For James Bond fans around the world, the number "007" tells a story all its own, signifying the derring-do associated with the intrepid secret agent invented by the pen of Ian Fleming and romanticized by dozens of popular film adaptations. For James Baker Jr., the number that begins his story of adventure, though of a seemingly more restrained sort, is "003."

Baker, who in 1971 went from his comfortable middle-class youth in the Chicago suburb of Oak Park to equally comfortable Williams College, nestled in the bucolic, purple, rolling hills of the Berkshires, remembers the day his mother called his dorm room to tell him what she'd just learned about his place in the nation's draft lottery. "Your number is 003," she said. "You're going to be killed."

Since then, those first 12 years with the Army in the Washington, D.C., area have evolved into a rather extraordinary saga — one that even the highly inventive Ian Fleming would find in many ways astounding. The plot twists are not minor — and they are not fiction. They involve the real stuff of life and death, but life and death on a dramatic scale that the young Jim Baker could never have envisioned as he contemplated his future career as a doctor. It was his lot to watch hundreds of young men (and older Army generals), and then eventually women, die in the prime of their lives of a disease no one had ever heard of — AIDS. "All my patients died," he says, and then finds the positive filter he seems to use to help maintain his steady focus on the work ahead: "It was a good experience to go through as a doctor; it defines you as a physician and gives you a healthy respect for nature and how tenuous life can be."

One of the men he watched die had helped save Baker's own life when they were part of a group of soldiers on a misguided training exercise in Virginia. Due to a commander's error, Baker and his fellow trainees had been put in a life-threatening situation in a swamp, and this man had gotten them out. "He was a gutsy guy and the commander's aide," Baker says. "He was also exactly my age. Four years later he came down with Kaposi's sarcoma."

"YOUR NUMBER IS 003," HIS MOTHER TOLD HIM. "YOU'RE GOING TO BE KILLED."

It was a time when there were no college deferments, and the war in Vietnam was still raging, so an immediate call-up loomed large for the young Baker. Fortunately for him, Army Reserve duty delayed his going to Vietnam, and Saigon fell to the Vietcong a few months before his college graduation.

But while America's costly involvement in the Vietnam War was over, Baker's life was changed forever. Having options for duty in the Reserves post-Vietnam, and with his scientific aptitude and interest in medicine already clearly identified, Baker began (at the behest of the U.S. Army) the first 12 years of his medical life. This involved medical school at Loyola Stritch and a residency in internal medicine at Walter Reed. A clinical fellowship in immunology and research at Walter Reed and the National Institutes of Allergy and Infectious Disease followed. After completing the fellowship, he returned to Walter Reed to participate in the transplant and HIV programs there.

It was also Baker's lot, since he was still in the Army Reserves, to be called back to duty for the Gulf War and to then find himself contemplating the possible deaths of thousands of the young men and women sent off to fight a war there. Here the issue was not gunfire but the more silent and invisible killers made from deadly germs and chemicals. "I'd go into a meeting and they'd be talking about 100,000 burn victims or 10,000 inhalation injuries," he remembers vividly. "It forces you to want to make a difference."

It's not that Baker has always sought the monumental personal or professional challenges that have come his way. In the early 1980s he changed his specialty from oncology to immunology just because he couldn't stand to administer any more of the extreme doses of anti-cancer drugs that were being used in clinical trials at the time. "I watched exceedingly toxic reactions from one particular drug, adriamycin, ➤

which was administered into almost every imaginable portal. It was so disturbing I still can't make red Kool-Aid (the color of the drug) for my daughter," he says. But such have been the powerful twists of fate to which he has been subjected that a member of his own family was administered the very same drug, in more limited dosages, for breast cancer less than two years ago.

These world events, unpredictable and jolting, on both a professional and a personal scale, have shaped Jim Baker. Part of him is still the teen-ager dazzled by laboratory work, the young man with a mind open to every possibility, always scanning the data ("I like data much better than hypotheses.") for the new ways of thinking that might be suggested.

Part of him is ancient bearded philosopher. He uses the words "tenuous" and "ephemeral" to describe the precariously delicate nature of our lives with an authority that few people can muster. He does not understand those who worry about not having tenure, those who think that a secure life can be imposed by formal structures. But his ability to distance himself in a thoughtful way from the world of medicine still has its limits. Supporting his immune-deficient patients who have what he calls the "real" problems keeps him firmly grounded in the here and now, and he remains troubled by the death last year of his mother from Graves' disease, finding it hard to let go of the notion that small corrections in how she was treated could have changed her outcome.

Victory Site: Last December Tarek Hamouda, Amy Shih and Jim Baker traveled to a remote military station in the Utah desert. There they demonstrated for the U.S. Army Research and Development Command the amazing ability of non-toxic nanoemulsions (petite droplets of fat mixed with water and detergent) developed at Michigan to wipe out deadly anthrax-like bacterial spores. The square vertical surfaces shown here were covered with bacterial spores; Michigan's innocuous nanoemulsion was most effective in killing the spores even when compared to highly toxic chemicals.

"I'D GO INTO A MEETING AND THEY'D BE TALKING ABOUT 100,000 BURN VICTIMS OR 10,000 INHALATION INJURIES," HE REMEMBERS VIVIDLY. "IT FORCES YOU TO WANT TO MAKE A DIFFERENCE."

Part of him is hardened soldier, although Baker suggests with a wry smile that "hard-headed bureaucrat" would be a more apt description. Still, he admits to understanding clearly that victory will go to the side that is best prepared, to the side that has the best resources, to the side that is least naive and most importantly to the side that has the greatest resolve. "I'm not the smartest person in the University of Michigan Health System," he says, "but I am among the most determined." These qualities have helped him become a savvy grantsman since arriving at the University in 1989. "I sort of knew where I needed to go" is how he modestly describes his ability to find the funding he needs — now totaling an astounding $20 million in projects currently underway at Michigan.

Baker had been at Michigan only for a year and a half when he was called away from his faculty position to go off to Desert Storm, a wrenching change of venue for someone just getting settled into the academic world. Everything since that time, he says, has felt "like a second chance, an opportunity that I am very lucky to have."

He is not letting the opportunity go to waste. A serendipitous twist, one not in the harrowing category of so many of Baker's life turns, happened when he was called in to consult about a patient who, as it turned out, was suffering from a severe drug allergy. The patient, who survived, and the doctor who helped keep him alive established a bond. He was, by chance, a retired Dow Chemical executive. Hearing about Baker's new work with tiny virus-like lipids and their ability to kill bacteria, he told him about the work of Dow chemist Donald Tomalia, who was just then making something he described as "a new form of matter"— extremely small nanoballs of nylon. The meshing of Baker's and Tomalia's ideas was so complementary that the two men were soon working together, and Tomalia joined Baker's team in the Center for Biologic Nanotechnology at Michigan last year. The nano-scale work being conducted there, where the organic and the synthetic meet on a level not even imaginable a short time ago, is opening up a new world of medicine.

While the work is smaller than small, the extraordinary possibilities for the future are allowing Baker's visions for the future to grow larger and larger. For "003," life in the fabulously adventurous world of nanomedicine promises more excitement than Ian Fleming imagined in his most inventive moments. m

MICHIGAN'S **RESEARCH** POWER

Total R&D Expenditures for Fiscal Year 1998

University of Michigan: $497 M

UCLA: $447M

University of Wisconsin: $444 M

University of Washington: $432 M

University of California, Berkeley: $420 M

University of California, San Diego: $419 M

Massachusetts Institute of Technology: $413 M

Johns Hopkins University: $411 M

Stanford University: $410 M

Texas A&M: $394 M

(Source: National Science Foundation; total excludes R&D expenditures for the federal Applied Physics Laboratory at Johns Hopkins University)

Top Ten Academic Institutions Ranked by Article Citations

1. Harvard University
2. Stanford University
3. California Institute of Technology
4. Yale University
5. University of Michigan
6. Massachusetts Institute of Technology
7. University of California, Berkeley
8. University of Washington
9. University of California, Santa Barbara
10. Cornell University

Top-Cited Fields of Study at the U-M in order of frequency of appearances, with Medical School fields noted in boldfaced type: education, psychology/**psychiatry**, astrophysics, **immunology**, computer science, **pharmacology**, economics/business, law, materials science

(Source: Institute for Scientific Information; based on frequency of appearances in 21 scientific fields, 1993-97)

Donald Tomalia, polymer chemist and scientific director of the Center for Biologic Nanotechnology, with Jim Baker. The pair were prominently featured in an article on the promise of nanomedicine in the January/February, 2000, issue of *Technology Review*, a publication of the Massachusetts Institute of Technology.

Faculty Serving on National Boards and Commissions:

Harvard University: 95

Massachusetts Institute of Technology: 69

Stanford University: 68

University of California, Berkeley: 57

University of Michigan: 51

University of Washington: 49

Cornell University: 40

University of Wisconsin: 39

University of Colorado, Boulder: 37

UCLA: 36

(Source: Survey conducted by U-M Office of the Vice President for Research)

The National Institutes of Health have nearly doubled funding awards for medical research at the **University of Michigan Medical School** in the past 11 years. The Medical School now ranks **9th** in the nation among all academic research institutions, public and private, and **3rd** among public universities in total grants from the NIH.

All information courtesy of the University of Michigan Office of the Vice President for Research.