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Thousands of Americans each year suffer serious damage to their delicate corneas. A BUSM research team is working to develop a radically different artificial cornea that may overcome common problems associated with existing devices. See story page 3.

Researchers unravel molecular structure of key protein in cholesterol delivery

A team of researchers in the Cardiovascular Institute of Boston University School of Medicine has succeeded in unraveling the genetic code, and thereby the structure, of an extraordinarily long protein molecule that is responsible for the majority of work in transporting cholesterol from the bloodstream to cells where it can be used.

Scientists have been working on the amino acid sequence of this protein, known as apolipoprotein B (apoB), for the past 25 years, but had only succeeded in identifying a string of 100 or so of its amino acid building blocks. In the last 18 months, a team headed by Vassilis Zannis, Ph.D., an associate professor of medicine and director of the Section of Molecular Genetics at BUSM, has succeeded in sequencing the entire 4,560 amino acids that make up apoB by using recombinant DNA techniques and computer analyses.

ApoB's significance lies in its role as a transporter of the cholesterol that circulates in the bloodstream. This cholesterol is obtained largely from food consumed; some also is synthesized by cells. Cells use cholesterol for such purposes as building membranes and, in some cases, hormones.

The movement of cholesterol from
continued on page 5

Gennaro Carpinito, M.D., center, and Christine T. Carr, R.N., administer autolymphocyte therapy to patient. The therapy is done on an outpatient basis. (Photo by Gustav Freedman)

Early experience with 'customized' cancer therapy proves promising

A Boston University School of Medicine group has developed a new cancer therapy in which the patient's own immune cells are temporarily removed from the body, immunized to the cancer, and then injected back into the patient.

Early experience with this "customized" type of therapy, called autolymphocyte therapy, shows that it can be done on an outpatient basis, has minimal side effects, and, most crucially, appears to prolong the lives of at least some of the cancer patients treated.

Autolymphocyte therapy was developed by Michael E. Osband, M.D., an associate professor of pediatrics at BUSM and director of the School's Clinical Immunotherapy Program, and colleagues Gennaro Carpinito, M.D., clinical director of the program, and Robert J. Krane, M.D., professor and chairman of the BUSM Department of Urology and chief of urology at the University Hospital.

continued on page 2

Cancer therapy...
continued from page 1

The basic goal of the first studies done with autolymphocyte therapy in 1982 and 1983 was to establish whether it is feasible and safe, not whether it works, said Osband. Nevertheless, even though most of the first patients treated had a particularly lethal form of cancer, metastatic kidney disease, a significant number did much better than would normally be expected.

"Studies for the past 50 years have shown that patients with metastatic kidney cancer have a very poor prognosis," said Osband. "On average, about half are expected to die within six months." Yet the researchers reported that, of 16 patients who lived long enough for the therapy to take effect, five experienced substantial shrinkage of their tumors. In four other patients, the cancer stopped growing for long periods of time.

"One patient has lived more than three years following treatment, in another case about two years, and in a third about 18 months," said the investigator, who also directs BUSM's Laboratory of Cellular Immunology and is head of BUSM's and Boston City Hospital's Division of Pediatric Hematology-Oncology. The researchers since have begun treating a second group of patients, and several of them also have experienced reductions in tumor size.

Autolymphocyte therapy is based on the theory that the cancer spreads in some patients because the body's immune system does not attack the tumors effectively. This may be due to problems with certain kinds of white blood cells called T-lymphocytes, or T-cells--so-called because such cells reach maturity in the thymus gland. Certain types of T-cells, such as helper T-cells or killer T-cells, can bolster the body's defenses against tumors, Osband said. But another group of T-cells--the suppressor T-cells--may sometimes undermine these defenses.

Michael E. Osband, M.D., developed the new cancer therapy, which appears to prolong the lives of some patients. (Photo by Bradford F. Herzog)

The normal role of suppressor cells is to inhibit immune responses, explained Osband, and it is likely these cells are simply carrying out that traditional mission in some cancer patients. "What may happen is that the immune system fails to recognize the tumor as foreign," he said, "so there is no immune response, or if the body does initiate reaction in the immune system, the suppressor cells cut off any budding response."

One piece of evidence supporting this theory is the fact that the number of suppressor T-cells is increased in many cancer patients. The aim of the BUSM group's therapy is both to help the immune system respond to the tumor, while at the same time reduce the activity of the suppressor cells.

The process begins with the removal from the blood of a small percentage of the body's white cells. The BUSM group stimulates this first group of patient cells to act as a kind of customized factory, producing, for that particular patient, a group of immune-system chemicals called mediators. Among them are such well-known mediators as interferon, interleukin 1 and interleukin 2, whose roles include activating immune-system cells like the helper T-cells.

"We think one important advantage of our approach is that we're using mediators produced by the patient's own cells, instead of injecting a version of interleukin 2 or some other mediator that was created through genetic engineering," said Osband. "That makes it a more specific and potentially less toxic regimen."

With the patient's own mix of mediators in hand, the group then draws more blood from the patient and creates a lymphocyte culture that contains the patient's mediators, an extract made from the patient's tumor, and the patient's own serum, the liquid portion of the blood with its red and white cells removed.

Prior to every treatment, a new group of white cells is removed from the patient and incubated in such a culture. Before the incubation starts, however, the investigators first eliminate as many suppressor T-cells as possible from the collected white cells, a task readily carried out using existing biochemical techniques that they have developed.

"In eliminating most of the suppressor cells, we think we're permitting the other T-cells to be more readily activated," said Gennaro Carpinito, M.D., who also is an assistant professor of urology at BUSM.

After the patient's white cells have been incubated for three days, they are reinjected into the patient. The BUSM group also puts patients on a widely used drug called cimetidine. The drug, usually employed to treat bleeding ulcers, works by blocking suppressor cells, said Carpinito. It is included in this therapy to further boost the immune system's ability to attack tumors by decreasing suppressor cell activity.

Although the BUSM group does not know exactly how the reinjected white cells and the cimetidine work, they suspect that the combination produces a true, long-lasting change in the patient's immune system. "On average, we're seeing a response

roughly six months following treatment," said Carpinito. That finding suggests that the treated lymphocytes are themselves not destroying the tumor, but work by educating other parts of the patient's immune system. The result is an immune system fully mobilized to attack the tumor.

Drawing on their early experience with the therapy, the BUSM investigators made some changes when they began treating their second group of patients last spring. For example, they are trying to destroy still more suppressor cells before reinjecting the white cells.

The current clinical trial will end early this year, with about 40 patients having been treated. The majority of those enrolled have been kidney cancer patients, although some will have cancer of the colon or pancreas or will have been been afflicted by a virulent form of skin cancer called malignant melanoma.

While it is too early in the second phase for clear cut results to have emerged, Osband said that some of the patients treated have shown highly encouraging responses.

Meanwhile, the BUSM team is analyzing blood samples from patients, in the hopes of shedding light on one of the key questions about the therapy: why some patients respond while others do not.

"By limiting autolymphocyte therapy to those patients in whom it is likely to work," he said, "we might get a response rate of 80 or 90 percent."

--Richard P. Anthony

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BUSM researchers strive to develop radically different artificial cornea

Thousands of Americans each year suffer serious damage to their corneas, the thin, transparent outer coating of the eye that is vulnerable to certain diseases and such traumas as chemical burns.

There now are two basic treatment alternatives for patients with corneas that have been severely damaged: a corneal transplant, or implantation of an artificial cornea. Both alternatives have advantages and disadvantages, according to Vickery E. Trinkaus-Randall, Ph.D., a research assistant professor of ophthalmology and biochemistry at Boston University School of Medicine.

Although a transplanted cornea can offer a long period of restored vision, the grafts often become cloudy and immunologic rejection occurs. Another problem associated with transplants, however, is that there are simply not enough donated eyes available. New corneas are being transplanted in Massachusetts at the rate of about 562 a year, but the rate would be much higher if more donated eyes were available, explained Trinkaus-Randall.

"Conditions that affect the cornea severely enough to require corneal transplantation include corneal ulceration, virus, bacteria, fungi, keratoconus and certain dystrophic corneal diseases," said Trinkaus-Randall. The cornea also is vulnerable to trauma, she noted, with thermal and chemical burns being two of the most common sources.

As for artificial corneas, or keratoprostheses, the main problem has been extrusion. "Because there is no 'healing,' surgical manipulations must be employed to secure the prosthesis," explained the investigator. "Despite the surgical manipulations, these devices have failed because of necrosis of the adjacent tissue, inflammation and epithelialization of the anterior chamber, where the epithelial cells migrate down and under the prosthesis and proliferation occurs, causing extrusion of the device." This and other problems associated with artificial corneas have prevented them from enjoying widespread use. A minimal number of artificial corneas are being implanted each year, which is far below the potential demand, said Trinkaus-Randall.

The investigator and her associates, however, are seeking to develop a radically different type of artificial cornea that may overcome the extrusion problem. The prosthesis will comprise a central transparent disc (hydrogel polymer) and a surrounding porous skirt (blown microfiber): The implant would replace the defective part of the native cornea and would be covered by an intact epithelial layer that is contiguous to the surrounding epithelium. It also would allow for "healing," whereby stromal keratocytes migrate into the surrounding skirt material, proliferate and synthesize basement membrane components.

"We need a type of material that is porous enough so that cells from the stroma, which comprises the main thickness of the cornea, can penetrate it," the researcher explained. "The resulting bond between the cornea and the prosthesis would block the downward growth of epithelial, or outer-layer, corneal cells, and thus prevent extrusion."

The BUSM research team, working with materials developed by polymer chemists at the 3M Company in Minnesota, already has solved many of the problems involved in designing such a system. This meant developing an artificial material that would be hospitable to the new cells and capable of surgical manipulation. In addition, said Trinkaus-Randall, the material had to be capable of holding a sprayed-on layer of the key proteins involved in corneal growth, a process called lamination. "We had some materials

that maintained cell growth very well," she said, "but we rejected them because we couldn't laminate the proteins on them."

Creating a prosthesis that provides a reliable replacement for damaged corneas is a formidable challenge. According to the BUSM investigator, the first problem that she and her associates had to solve was creating an effective culture system for growing new corneal epithelial cells. The material that eventually proved most effective was a polyvinyl alcohol copolymer hydrogel, developed at 3M. The investigators found that disks of the material, which look and feel similar to soft contact lenses, provide an excellent bed for the growth and maintenance of epithelial cells.

Besides identifying a suitable material, however, the researchers also had to define the best conditions for culturing cells on the hydrogels prior to implantation. Parameters that were tested included surface type, length of culture time and number of cells added to the central portion. It also was necessary for the researchers to maintain a balance between growth and sloughing.

"As the cells grow and divide, some of them will slough off," explained the investigator. "If you don't maintain a rough balance between the ones that are growing and those that are sloughing off while you're culturing the cells, you get hyperproliferation of the cells and an erratic epithelial structure."

The technique devised by the BUSM group, which involves starting with a single layer of epithelial cells and culturing them for four to five days, has produced excellent results in laboratory tests. Once the disk with cultured cells is implanted, said Trinkaus-Randall, the cells continue to divide. The host need not be the source of the cultured cells, she noted.

"We've found that the epithelial cells we're implanting grow out beyond the disk onto host tissue, and the host epithelial cells grow onto the disk, they don't seem to be rejecting each other," she said.

Meeting the second of the research venture's major goals, creating tight bonds between the prosthesis and the native cornea, requires materials with qualities quite different from those that make the disk work, said the investigator. "What we're working on is a peripheral skirt, which will be attached around the outside of the disk," said Trinkaus-Randall. "While the disk is quite dense, this material is what's known as a blown microfiber and it's extremely porous." The porosity permits the influx of cells from the inner layer of the patient's cornea, she noted.

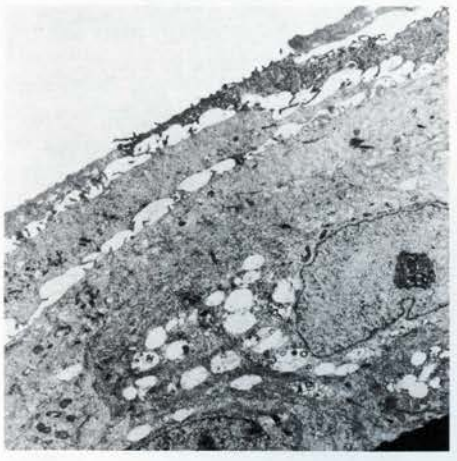
The material that the researchers currently are using is another polymer, a polybutylene. Trinkaus-Randall said that experiments show it allows the penetration of corneal keratocytes; not clear yet, however, is whether it meets another key prerequisite.

"The skirt must hold a suture, and the hole created by the needle must seal once you remove the suture," said Alexandra Vadasz, M.D., a research fellow in the BUSM Department of Ophthalmology. "If it tears when you try to put a stitch through it, it's not going to work as a long-term prosthesis."

Despite these hurdles, the researchers believe that the use of this unique keratoprosthesis is promising.

Other BUSM faculty involved in the research include Howard Leibowitz, M.D., professor and chairman of the Department of Ophthalmology, and Carl Franzblau, Ph.D., professor and chairman of the Department of Biochemistry.

--Richard P. Anthony



Transmission electron micrograph of corneal epithelial cells on the hydrogel after eight days of culture. Several layers of epithelium are present and cell junctions can be detected. (Photo courtesy of Vickery E. Trinkaus-Randall, Ph.D.)

Vickery Trinkaus-Randall, Ph.D., and her collegues seek to develop a radically different type of artificial cornea. (Photo by Bradford F. Herzog)

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Cholesterol ...
continued from page 1

blood plasma to the cells and back to the plasma is accomplished by the action of such proteins as apolipoprotein B, apolipoprotein E and apolipoprotein A-1. Zannis and his team found that apoB forms a complex with cholesterol and other lipids in the bloodstream. This complex is known as low density lipoprotein (LDL) or "bad" cholesterol. According to the researchers, LDL contains a single apoB molecule and 2,000 cholesterol molecules. High density lipoprotein (HDL) or "good" cholesterol, another type of lipid protein complex, is involved in the removal of cholesterol from the cells. *(Please refer to diagram, page 6.)*

ApoB ferries the cholesterol to the surface of cells where it binds to another protein, called the LDL receptor. "In this regard, apoB serves as the key and the LDL receptor serves as the lock," explained Zannis. Following binding, LDL is taken into the cell where apoB is destroyed and the cholesterol is released to be utilized by the cell as required.

What concerns Zannis and his group is what happens when the apoB-LDL receptor system breaks down, causing a harmful increase in blood cholesterol. "The buildup of cholesterol may occur as a result of genetic changes in one or the other protein," said Zannis. "The interaction or 'locking in' of apoB and the LDL receptor is inefficient and the cholesterol, rather than being delivered to the cell, builds up in the bloodstream." This increase in cholesterol clogs the arteries and increases the risk of atherosclerosis and heart disease.

Once the molecular structure of apoB was identified, the researchers fed the information to a computer, which identified the probable regions of apoB involved in binding to the LDL receptor. "The regions we identified by computer still have to be verified by laboratory experiments, but we think that a number of people with high levels of cholesterol in their blood may have had changes in that part of the apoB protein. Now that we know the normal structure of the protein, we know what to look for," said Zannis.

Computer analysis of the apoB protein was performed by David Atkinson, Ph.D., a BUSM associate research professor of medicine and biochemistry. This analysis revealed several possible regions that may have to do with recognition of receptor sites. One region in particular has a much higher positive charge than the rest of the molecule and is surrounded by regions that also exhibit a positive charge, although to a lesser degree.

"It is possible that several regions of apoB must be together for the molecule to be recognized by the LDL receptor," Zannis hypothesized. "So far, it has been found that if you destroy the positive region of apoB it will not be recognized by the LDL receptor."

A BUSM research team headed by Vassilis Zannis, Ph.D., right, and Christos Cladaras, Ph.D., principal collaborator in the research, unraveled the genetic code of the protein molecule apolipoprotein B (apoB) that transports cholesterol in the bloodstream. The team used recombinant DNA techniques and computer analyses to sequence the 4,560 amino acids that make up apoB. (Photo by Bradford F. Herzog)

Zannis said that this new understanding about the structure of apoB will enable physicians to screen patients with high cholesterol for changes in the apoB gene and apoB protein. Early identification of this problem and proper treatment with drugs or dietary programs may prevent or delay the development of heart disease and extend the life of these patients.

Even more important, however, will be the ability to identify molecular markers in suspected carriers of this mutation, for instance, in people with a family history of elevated blood-cholesterol levels, and to screen them as early as birth or one year.

"If you find markers that indicate this individual will have high blood cholesterol, then you can start preventive measures right away, before any damage is done. If blood tests of an adult male, for example, show a level of cholesterol above 300, then the damage already has been done to the blood vessels and all you can hope to do is to minimize the effects," said Zannis.

Zannis and his group also will be looking at new ways to control the production of apoB in the body. "If there are high levels of LDL in the bloodstream, there also must be high levels of apoB because apoB is an essential part of LDL. Our idea is that if one has lower levels of apoB available, there may be less of an accumulation of LDL in the blood and the body will find other ways of eliminating the excess cholesterol," explained the researcher.

The researchers have been able to start synthesizing apoB in vitro using the blueprint of the gene and can now start experimenting with drugs that interrupt or slow down its production.

"The hope is that if we understand

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the structure and action of all the proteins involved in cholesterol transport and the regulation of their synthesis, then we'll be in a position to describe at the level of simple molecules all the diseases that cause high cholesterol in the blood. We will then have the tools to diagnose the condition and start treatment before it's too late."

Other members of the BUSM team that decoded the apoB structure in record time include: Christos Cladaras, Ph.D., a research instructor in medicine and principal collaborator in the research; Margarita Hadzopoulou-Cladaras, Ph.D., a research fellow in the Section of Molecular Genetics; Robert T. Nolte, a graduate student in BUSM's Biophysics Institute; and Rafael Avila, M.D., a colleague from the Harvard School of Public Health.

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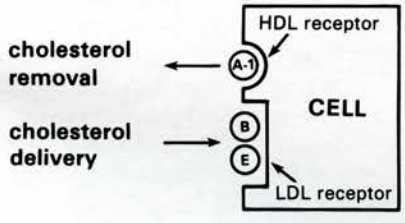
--Caroline H. Lupfer

Cellular Cholesterol Homeostasis



Schematic representation shows cholesterol being transported in and out of a cell. 'B' represents LDL, a complex made up of lipids and apoB. 'E' represents apoE containing lipoproteins. 'A-1' represents HDL or apoA-1 containing lipoproteins. 'B' and 'E' deliver cholesterol to the cells and 'A-1' removes cholesterol from the cells. Breakdown of this process may cause the accumulation of cholesterol in the bloodstream and result in clogged arteries. (Illustration by Educational Media, BUSM)

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