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Monoclonal antibodies are being used experimentally in the diagnosis and treatment of cancer and other diseases. School of Medicine researcher Jacqueline Sharon, Ph.D., is working to improve the design of these "magic bullets." See story on page 3.

Form of vitamin D may offer hope for psoriasis sufferers

A Boston University School of Medicine researcher is experimenting with a form of vitamin D not presently available to consumers as a possible safe and effective therapy for the stubborn clinical problem of psoriasis, and is achieving results that bode well for the more than 50 million people around the world who suffer from the skin disease.

A disorder of unknown cause, psoriasis afflicts some 1 to 3 percent of the total world population and is characterized by excessive production of epidermal cells, producing patches of thick scales that are itchy, burning, prone to bleeding and unsightly. Currently, there is no totally effective treatment or cure for the disease, and the therapies that do exist, such as exposure to ultraviolet light or steroid medications, often are ineffective after long-term use or possibly even toxic.

Michael F. Holick, Ph.D., M.D., a professor of medicine and director of the newly established Vitamin D, Skin and Bone Research Laboratory and director of the General Clinical Research Center at BUSM, is experimenting with the active form of vitamin D as a possible safe and effective therapy for psoriasis. Vitamin D, which is obtained from the diet or as a result of exposure of the skin to sunlight, is activated in the liver and kidney to form 1,25-dihydroxyvitamin D_3.

As an endocrinologist who

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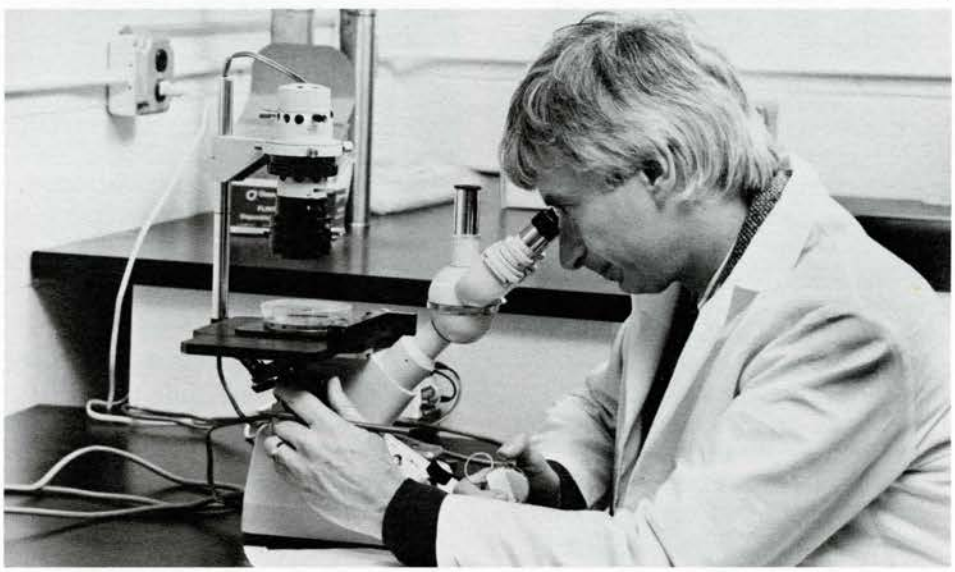

Michael F. Holick, Ph.D., M.D., is experimenting with a promising new therapy for the stubborn clinical problem of psoriasis. (Photo by Gustav Freedman)

BUSM researchers test new treatment to reduce pneumonia in hospital patients

One of the most serious problems confronting hospitals is nosocomial, or hospital-acquired, infections in patients.

On average, 5 to 10 percent of hospitalized patients develop such infections, said Donald E. Craven, M.D., an associate professor of medicine at Boston University School of Medicine. Of these hospital-acquired infections, pneumonia is the most serious.

"Nosocomial pneumonia, which accounts for about 15 percent of all nosocomial infections, is the second most common hospital-acquired infection in the United States," said Craven.

Although less common than urinary tract infections, nosocomial pneumonia is associated with higher morbidity and mortality rates, said the infectious disease specialist. Mortality rates are greater than 50 percent for patients who develop pneumonia in the medical or surgical intensive care unit. Moreover, he noted, the development of pneumonia in the hospital adds another unnecessary burden to a patient who is seriously ill.

Most efforts aimed at reducing the toll exacted by nosocomial pneumonia have centered on the use of new antibiotics, said Craven. "The perception

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has been that there's very little we can do to prevent nosocomial pneumonia, so the emphasis has been on trying to develop new types of antibiotics to treat it," he said.

According to Craven, a wide range of factors has been blamed for the high rate of pneumonia among hospitalized patients. Patients who are very ill, for example, have weakened immune systems. And despite advances in techniques for minimizing infections, hospitals still have difficulty preventing the spread of infectious agents.

Now, however, Craven and his associates have identified a new strategy for preventing pneumonia in intensive care unit patients who need mechanical ventilators in order to breathe. In a recent report published in the *New England Journal of Medicine,* the group said that maintaining the patient's normal stomach acid may hold the key to reducing the incidence of pneumonia.

Craven's specific concern is the medication routinely used to prevent gastrointestinal bleeding among patients in intensive care units. "Virtually all patients who enter an intensive care unit get regular antacids that neutralize gastric acid, or therapy with histamine type 2 (H2) blockers, which block gastric acid secretion," he said. "The purpose of the therapy is to reduce the risk of gastrointestinal hemorrhage, which is a serious problem in patients who are critically ill."

In reducing levels of gastric acid, however, there is overgrowth of bacteria in the stomach. These may reflux into the throat and be aspirated into the lung, resulting in the pneumonia.

"Four years ago," said Craven, "our group did a study here at Boston City Hospital in which we analyzed the risk factors for nosocomial pneumonia among intensive care patients who needed mechanical respirators to breathe. One of the risk factors most strongly associated with pneumonia was drugs that block or neutralize gastric acids."

According to Craven, this initial study has had little impact on hospital treatment of intensive care patients because, until recently, there has been no alternative therapy to antacids or H2 blockers. Since that study, however, sucralfate, a new form of medication for preventing such bleeding, has been developed. Sucralfate works by creating a protective coating in the stomach rather than by reducing gastric acid levels. It was the development of such an agent that prompted Craven and Michael Driks, M.D., an infectious disease fellow, to perform the study, which recently was published.

"We wanted to see whether we could reduce the incidence of pneumonia by using sucralfate instead of the usual regimen of antacids or H2 blockers," said Craven. "We felt that maintaining relatively high levels of gastric acid would decrease the growth of pneumonia-causing bacteria in the stomach, and the result would be fewer cases of pneumonia."

The patients selected for the study were critically ill, intensive care unit patients who required the aid of mechanical ventilators. The types of problems that had brought the patients to the hospital included heart attacks, head trauma, drug overdoses and severe respiratory disease.

Of the 130 patients enrolled in the study, roughly half were randomly chosen to receive conventional treatment with H2 blockers or antacids or both, while the rest received sucralfate.

The specific type of bacteria the researchers were interested in are called gram-negative bacilli. These rod-shaped organisms have been pinpointed as the major culprits causing hospital-acquired pneumonia.

Donald E. Craven, M.D., shown with a patient, and his colleagues are studying the effects of a new drug for reducing the incidence of hospital-aquired pneumonia in critically ill patients. (Photo by Bradford F. Herzog)

"We looked at the rates at which these bacilli were present in both the stomach and the pharynx of the two treatment groups," said Craven, "and we found that the levels of pneumonia-causing bacteria were often several orders of magnitude higher in patients receiving H2 blockers or antacids compared to patients given sucralfate."

The investigators found that the rate of pneumonia in the antacid/H2 group was nearly double that of the sucralfate group (23 percent as compared to 12 percent).

Craven said the data suggest "that sucralfate appears to be as effective as H2 blockers and antacids at preventing stress bleeding, but is associated with a much lower rate of nosocomial pneumonia." He predicted that his group's study, when combined with similar findings from other medical centers, is likely to have a major

impact on the management of stress bleeding in intensive care patients.

In addition to Driks, other associates at Boston City Hospital who have been instrumental in the project's success include: Bartolome R. Celli, M.D., an assistant professor of medicine at BUSM and a pulmonary specialist; Harrison W. Farber, M.D., an assistant professor of medicine; Marie Manning, a medical technician; William R. McCabe, M.D., a professor of medicine; and Suzanne A. Wedel, M.D., an assistant professor of surgery.

—Richard P. Anthony

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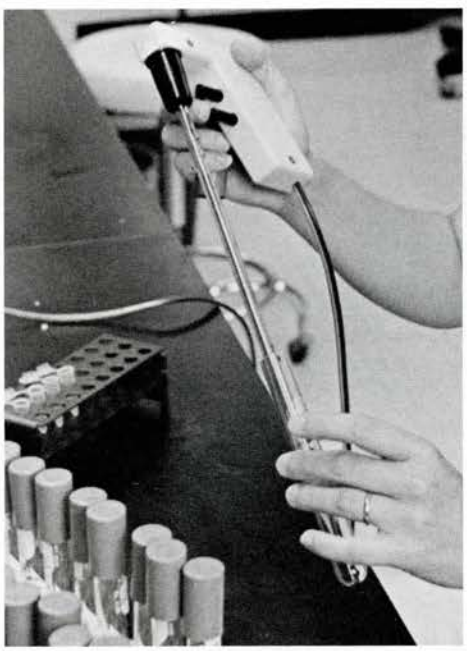
Building better monoclonal antibodies subject of BUSM researcher's efforts

Monoclonal antibodies represent one of the most exciting areas of basic research under investigation today. As specially designed "magic bullets" that can seek out and bind to specific targets with great efficiency, they are being experimented with in both the diagnosis and treatment of various diseases, particularly cancers. Yet, because no one fully understands the molecular interactions between natural antibodies and their target antigens, there is room for improvement in the design of new monoclonal antibodies.

Since joining Boston University School of Medicine in 1985, Jacqueline Sharon, Ph.D., an assistant professor of pathology and biochemistry, has been working on the problem of how to build a better monoclonal antibody. The clinical applications of her work someday may include the eradication of tumors and the control of the rejection of transplanted organs.



In developing more efficient monoclonal antibodies, Sharon first must grow them in bacterial plasmids. Here, Sharon transfers bacteria containing antibody genes. (Photo by Gustav Freedman)

Normally, we defend ourselves against disease with the help of antibodies, specialized proteins produced as needed by certain cells in the body to neutralize foreign substances, or antigens. Even under normal conditions, however, not all types of antibodies attack and bind to target antigens with 100-percent efficiency. There may be factors in an antibody's gene structure that make it less efficient than it could be.

One reason for developing monoclonal antibodies is to overcome the binding deficiency of natural antibodies and produce, in the laboratory, a pure strain of highly specific antibody that will react strongly with a particular antigen. However, because monoclonal antibodies today are made by first raising the antibody of choice in a mouse and then fusing it with a tumor cell line to produce a clone of that antibody, there may be a problem with rejection when the new antibody is introduced into another species, such as a human, to do its work.

"Most experimental antibodies are from mouse systems rather than from humans for ethical reasons and because human cell lines cultured for antibodies lose their productivity and die out after a while," explained Sharon.

In order to design the most effective antibody and to overcome the tendency for rejection, Sharon is investigating which areas of an antibody molecule are crucial to its operation and which are not. In her experiments, she introduces mutations into a gene responsible for producing an antibody

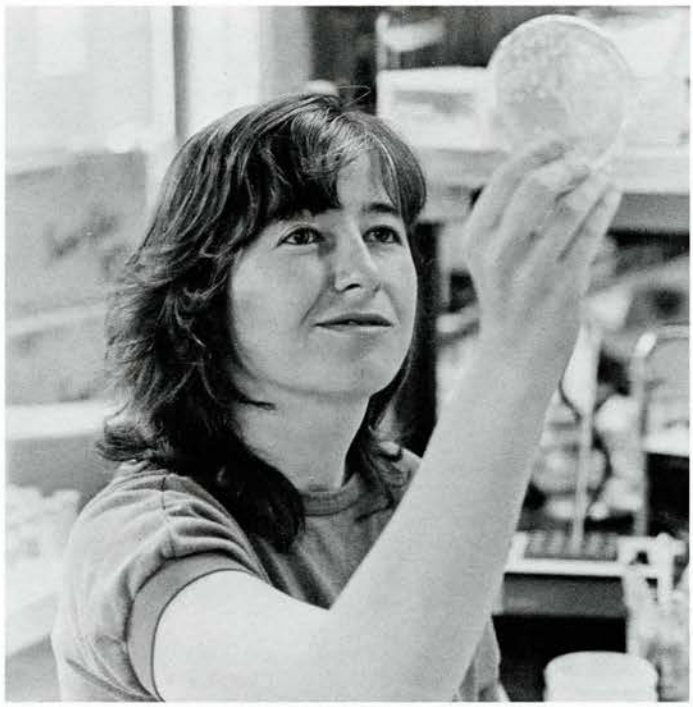
and observes how those changes affect the antibody's structure and, therefore, its ability to bind to the target antigen.

She uses a technique called oligonucleotide-directed mutagenesis that allows her to direct whatever changes she wants by making specific alterations in the chemical composition of the gene. Sharon then measures the binding constant—the strength of the interaction between the antibody and the antigen. Her goal is to find out if there are certain positions in the antibody gene sequence that are especially important to the binding constant. If so, it may be possible to manufacture an antibody with an optimum binding ability for a particular antigen.

In her current studies, which are funded by the National Institutes of Health and the Whitaker Foundation, Sharon is looking at two types of antibodies that bind to the same antigen, one of which has a binding efficiency 200 times greater than the other. A comparison of the antibodies' amino acid sequences has revealed eight differences in one area—called the heavy chain portion—of the molecules. What Sharon wants to know is how many of these differences contribute to the stronger interaction.

"By using oligonucleotide-directed mutagenesis, you can ask how many of these differences actually contribute to turning the sequence of antibody X into antibody Y, and you can study them one by one. You can ask at each stage what is the effect of changing this particular amino acid," said Sharon.

According to Sharon, every antibody molecule has a variable region that actually does the work of binding to an antigen and a constant region that is not directly involved. Within the variable region, there are even smaller regions known as hypervariable regions that may be responsible for the optimal binding efficiency.



Jacqueline Sharon, Ph.D., is investigating what areas of an antibody molecule are crucial to its operation. (Photo by Gustav Freedman)

In one set of experiments involving a mouse-model system, Sharon plans to determine an antibody's optimal protein sequence for binding efficiency and then to graft the small hypervariable portion of that molecule into its framework and then into the constant region of another mouse antibody molecule to see if specificity to the target antigen is retained. Theoretically, this would work in humans, too.

"The idea is that if you take just the variable region of a mouse antibody and hook it to a human constant region, you won't get an immune response against the constant region when you put the whole molecule into a human for treatment," she explained. "And if you could put an even smaller part, the hypervariable regions, into a human constant region, you have an even better chance that there won't be an immune response."

In addition, Sharon is cloning DNA from a rat's variable region and fusing it onto a mouse antibody constant region to test the rejection response. Most of the work involved in research of this sort, she said, is in trying to get the antibody genes to clone in the first place. Sharon expects this fine-tuning to take the better part of a year before she has the newly designed monoclonal antibodies to test in the animal systems.

—Caroline H. Lupfer

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2. Sharon, J. *et al:* Site directed mutagenesis of an invariant amino acid residue at the V-D junction of an antibody. *Proc Natl Acad Sci. USA 83:* 2628-2631, 1986.

Psoriasis...
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specializes in bone metabolism, Holick is well aware of the multifaceted role vitamin D plays in the absorption and regulation of calcium in the body and the maintenance of healthy bone. In addition, Holick and his colleagues have been looking at the role the active form of the vitamin may have in the treatment of previously intransigent skin diseases.

The principal natural source of vitamin D lies not in food but in the exposure of skin to sunlight. High-energy, ultraviolet sunlight enters the skin and is absorbed by provitamin D_3, a precursor of cholesterol. A natural photochemical chain reaction converts these molecules to previtamin D_3, which in turn is converted through a thermal reaction in the epidermis to the more stable molecule, vitamin D_3. Vitamin D_3 enters and is carried by the circulation to the liver and kidney, where it is metabolized to the active, hormonal form—1, 25-dihydroxy-vitamin D_3.

Holick, who also heads the Osteoporosis Clinic of the Evans Medical Group at the University Hospital and is chief of the Endocrine Unit at Boston City Hospital, was one of the discoverers of this active form of vitamin D 16 years ago, and also was the first to chemically synthesize it in the laboratory for clinical use.

While it has been long understood that the skin produces vitamin D, Holick's group found that the skin also responds to the active form of the vitamin and does so in a very specific manner.

The active form of the vitamin, abbreviated as 1,25-$(OH)_2$-D_3, is a potent hormone for inhibiting proliferation and inducing terminal differentiation of human epidermal cells, explained Holick. Many tissues of the body, including the skin, have receptors for 1,25-$(OH)_2$-D_3. Given that psoriasis is a disease characterized by hyperproliferation of epidermal cells, Holick reasoned that if the receptors were there, then the hormonal form of the vitamin should act to prevent further proliferation and induce differentiation into normal skin cell types.

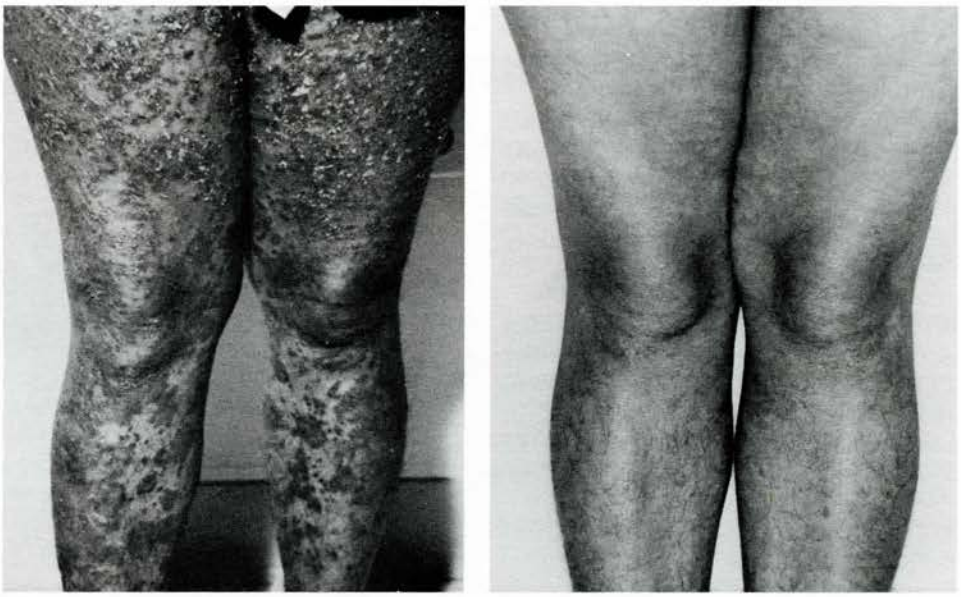

Photo at left shows a patient with psoriasis before the vitamin D treatment, and photo at right shows the same patient following the treatment. (Photos courtesy of Michael Holick, Ph.D., M.D.)

In a recent trial conducted in the fall and winter in Boston to see how 1,25-$(OH)_2$-D_3, administered either topically or orally, would work against psoriasis, Holick found excellent and rapid clearing of the skin in about 30 to 40 percent of the individuals studied. In another 30 to 40 percent there was a good, but not total, response, which continued to improve slowly over time. In some patients, however, neither application seemed to work. In these cases, the question became: What is it that keeps these individuals from having a good response? Cultured tissue samples from resistant patients showed that there were receptor sites for the active form similar in quantity and quality to those found in normal skin.

In his studies, Holick tested the effects of 1,25-$(OH)_2$-D_3 on cultured fibroblasts (cells from the dermis) and keratinocytes (cells from the epidermis) from psoriatic patients and found that the active form of the vitamin caused a dose-dependent inhibition of proliferation and induction of terminal differentiation similar to its effect on keratinocytes of normal skin. He then treated 17 patients with psoriasis vulgaris with orally or topically administered 1,25-$(OH)_2$-D_3.

In 10 of 14 patients who were treated orally, there was a significant clearing of the psoriatic plaques. Three patients had complete clearing while they were on the drug, whereas only four of the 17 received little or no benefit from the therapy. Of the three patients who received topically administered 1,25-$(OH)_2$-D_3, all showed a rapid response with complete clearing after six weeks of therapy.

The responsiveness of patients to the active form of vitamin D seemed to correlate with the *in vitro* response of the patients' cultured skin samples to the antiproliferative activity of the

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drug. In some of the patients treated orally, however, there were some reappearances one or two months after the therapy was discontinued. These lesions generally were smaller and were located in areas previously uninvolved, but also were slower to respond to a reapplication of the 1,25-$(OH)_2$-D_3.

One potential drawback in using 1,25-$(OH)_2$-D_3 is the possibility of hypercalciuria or hypercalcemia, an excess of calcium in the urine or blood, which could lead to kidney stones or kidney damage. This aspect was monitored carefully in Holick's study by measurements of concentrations of calcium in the blood and urine every two weeks. Measurements taken prior to therapy indicated that psoriasis is not caused by a deficiency of the active form of vitamin D. Furthermore, it was discovered that the patients could tolerate a single large dose (2.0 micrograms/day) at bedtime much better and with no toxicity than smaller doses during the day.

Based on these preliminary findings, Holick believes that orally or topically administered 1,25-$(OH)_2$-D_3 may be a safe and effective alternate therapy for the treatment of psoriasis. In addition, an evaluation of the responsiveness of cultured fibroblasts and keratinocytes from psoriatic patients to the antiproliferative activity of 1,25-dihydroxyvitamin D_3 may be a useful predictor of which patients will respond to this approach.

Since he began his studies using the active form of vitamin D, Holick has received letters from approximately 3,000 people eager to participate in further trials of the drug, which now are being carried out in BUSM's General Clinical Research Center.

"We think that the active form of vitamin D may be an important therapy alone, in combination with other treatments, or in the maintenance of remission for psoriatic patients," said Holick. Further trials are under way to test these hypotheses, and *in vitro* studies are in progress to determine the biological significance of the partial resistance to 1,25-$(OH)_2$-D_3.

—Caroline H. Lupfer

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