Tumor Immunology (II): Strategies for Cancer Therapy

The immune system, in the views of many scientists, is the body's principal defense against cancer. It acts in response to antigens present on the surfaces of cancer cells but not on normal cells and destroys the aberrant cells before they can proliferate. Cancer results when a defective immune system fails to perform adequately. If this theory is correct (and possibly even if it isn't), augmenting the body's capacity to mount an immune attack should be a workable strategy for preventing, controlling, or curing cancer.

The current emphasis is on controlling or curing cancer, rather than on preventing it. Although animals have been immunized against cancers, a vaccine for human use will not be available for many years, even by optimistic estimates, because of the numerous technical problems that remain to be solved. Meanwhile, investigators are exploring a number of immunotherapeutic strategies for treating cancer. Some are more advanced in terms of the extent and duration of clinical trials with humans, while others are still in the earliest stages of investigation. All are considered experimental techniques.

The experimental nature of immunotherapy raises bioethical questions for those testing it clinically. No one wants to deprive a cancer patient of the best treatment available. So immunotherapy is often used in conjunction with conventional therapies including chemotherapy—a fact that could complicate interpretation of the results. Alternatively, its use may be restricted to patients who have very poor prognoses, even with the best treatment. They include those suffering from acute myelogenous leukemia, osteogenic sarcoma, or recurrent melanoma, for example, or patients with advanced disease and large tumor burdens. Yet most investigators think that the best application of immunotherapy will be eradication of the relatively few tumor cells remaining after surgery, radiation, or chemotherapy; after such treatment the patient may be clinically free of the disease.

The immune system may be augmented actively if the patient (or his lymphocytes in culture) is directly exposed to stimulating antigens. These can be tumor-associated antigens (active specific immunotherapy), or they can be unrelated to tumor antigens (active nonspecific immunotherapy). The most extensively studied immunotherapies employ BCG (bacillus Calmette-Guérin) and are nonspecific. Bacillus Calmette-Guérin is an attenuated strain of the bacterium that causes bovine tuberculosis. It is frequently used in Europe, and occasionally in the United States, as a vaccine against human tuberculosis. BCG is generally thought to be a potent, nonspecific stimulator of the immune system.

The Use of BCG

Most data on the use of BCG for cancer therapy derive from studies on patients with melanoma. The primary lesions of melanoma are located in the skin where they are easily accessible for treatment and observation. Among the investigators who have used BCG for treating this cancer are Donald Morton of the University of California Medical Center in Los Angeles and Carl Pinsky and Herbert Oettgen of Memorial Sloan-Kettering Cancer Center in New York. They found that direct injection of BCG into cutaneous lesions usually causes regression, often complete, of the injected lesions. Sometimes uninjected lesions also regressed. Occasionally, patients treated with BCG have had complete remissions of their disease that have lasted for 2 or 3 years or longer. Melanoma, however, despite its usually poor prognosis, is unpredictable and remissions sometimes occur anyway. Metastatic melanoma lesions of the visceral organs or brain are not affected by BCG injections.

The side effects of BCG injection into lesions can be both unpleasant and dangerous. It usually causes inflammation and abscess formation at the injection sites. Pinsky said that more than 40 of the approximately 50 patients in their trial also had fevers lasting several days after the injections. Almost half the patients suffered from recurrent fevers with flulike symptoms including nausea and vomiting. Liver abnormalities were frequent.

The most dangerous side effect of BCG therapy is a severe hypersensitivity reaction caused by allergy to the organism in individuals who have had more than one exposure. Pinsky had to terminate the BCG therapy of one patient who had a severe reaction. This patient recovered. However, Charles McKhann of the University of Minnesota Medical School, Minneapolis, and Lynn Spitler of the University of California Medical Center in San Francisco, attribute the death of two patients treated with BCG to hypersensitivity to the organism and consequent shock.

Because of BCG's toxicity, many investigators are exploring the use of other nonspecific stimulators of the immune system that may be less toxic than living BCG. These include membrane fragments and extracts of BCG, a bacterium called *Corynebacterium parvum*, and a drug named Levamisole. Alternate methods of administering BCG such as scarification (the production of superficial scratches into which BCG is introduced) also produce less severe side effects than does injection into lesions.

Tumor regression induced by BCG may result from the activation of macrophages that attack the tumor cells. Herbert Rapp and Berton Zbar and their colleagues at the National Cancer Institute, Bethesda, Maryland, have developed a guinea pig model for investigating cancer immunotherapy. Both the immune responses of guinea pigs and the tumors studied (carcinomas) resemble those found in humans.

Rapp and Zbar immunized guinea pigs against one tumor line and then challenged them with intradermal injections of tumor cells. When the challenge cells were from a different line than that used for immunization, tumors grew at the site of injection. When they were from the same line or were a mixture of the two lines, inflammation occurred at the site of injection and tumors did not develop. According to Rapp, tumors did not form at the sites where the mixture was injected because the inflammatory response elicited by tumor cells of the line used for immunization also caused nonspecific destruction of the other tumor cells.

Macrophages appeared to play a role in this nonspecific cell killing. Rapp and Zbar observed large numbers of these cells at the sites of the delayed skin responses. They found that intradermal injection of the macrophages themselves caused inflammation that suppressed the growth of tumor cells subsequently injected into the inflamed sites.

According to Rapp and Zbar, BCG, which produces a strong inflammatory response at the site of injection, suppressed tumor growth when injected with tumor cells. It also induced regression of established tumors and even of early metastatic lesions in the lymph nodes. Larger, more advanced tumors, however, were less susceptible to the effects of BCG. Since Rapp and Zbar, with Michael Hanna of Oak Ridge National Laboratory, Oak Ridge, Tennessee, observed large numbers of macrophages in the lymph nodes of animals injected with BCG, they hypothesize that the immune system responds to BCG injection with sensitized lymphocytes that can recognize and react with BCG antigens. These lymphocytes then release a product called migration inhibitory factor that can immobilize macrophages in the injected tumors where they can destroy tumor cells. Tumor cell destruction releases tumor antigens which in turn provoke additional cell-mediated immune responses, now specific for tumor cells.

There is evidence that macrophages can destroy cancer cells in humans, too. Edmund Klein of Roswell Park Memorial Institute, Buffalo, New York, in collaboration with Isaac Djerassi of Mercy Catholic Medical Center, Darby, Pennsylvania, has used macrophage injections to treat a variety of cancers in humans. The tumors regressed but the treatment is probably not practical for large-scale application. Collection of macrophages is expensive and time-consuming. Furthermore, repeated injections of macrophages may provoke an immune response to the cells themselves, a problem shared with other potential therapies involving injection of cells collected from donors. Nevertheless, the investigators think that their findings constitute further evidence for the importance of macrophages in tumor destruction.

Specific stimulation of the immune system with tumor cells or tumor-associated antigens is another approach to immunotherapy now being explored in a number of laboratories. Much of this work is still in an earlier stage of development than that with BCG. It has been restricted to relatively few human patients and to animal models.

One way of achieving specific activation of a patient's lymphocytes is to culture them with his tumor cells. The sensitized lymphocytes can then be reinfused into the patient where—it is hoped—they will mount an attack on the tumor cells. McKhann has attempted this approach with four patients suffering from widespread metastatic melamona; only one of the four showed any improvement, and she did not become clinically free of disease.

Another possibility is in vivo stimulation of the immune system by using either tumor cells or isolated tumor antigens. Some investigators, such as J. George Bekesi of Mount Sinai School of Medicine, New York, and Richard Simmons of the University of Minnesota School of Medicine, think that the efficacy of tumor cells in stimulating immune responses can be increased by first treating the cells with the enzyme neuraminidase. This enzyme breaks down the sialic acid coating on the cells; this coat, which is heavier on tumor cells than on normal ones, may mask tumor antigens and thus help the cells escape immune surveillance.

Regression of Mouse Tumors

Simmons has found that a number of tumors transplanted into mice regress after injection of tumor cells treated with neuraminidase. The effect is tumor-specific: a tumor will not regress if the treated cells are from a different tumor line. The tumors also failed to regress if the challenge cells had been incubated with neuraminidase inactivated by heating. This indicates that the tumor cells do not provoke an effective immune response unless their antigenicity is increased by the action of the enzyme.

Active specific immunotherapy also increased the survival of mice with "spontaneous" leukemia, according to Bekesi. If untreated, 95 percent of the animals die within 8 weeks of clinical diagnosis. Immunotherapy with neuraminidase-treated leukemia cells in addition to chemotherapy greatly increased both the percent of animals that survived and the survival time of those that did die when compared to the effects of chemotherapy alone.

Bekesi, with James Holland of Mount Sinai School of Medicine, has begun clinical trials of immunotherapy with neuraminidase-treated leukemia cells for human leukemia. They first reduce the patient's tumor burden to a minimum with chemotherapy. Most investigators think that this step is essential because the immune system has a limited capacity to destroy tumor cells. According to Bekesi, their preliminary results suggest that immunotherapy in addition to chemotherapy increased the length of the patients' remissions by as much as 50 percent over chemotherapy alone. Moreover, when the patients relapsed, they could more readily be induced into additional remissions.

Immunotherapy can involve a combination of specific and nonspecific stimulation of the immune system. For example, Ray Powles and his colleagues at the Chester Beatty Institute in London are using leukemia cells plus BCG to treat adult patients for acute myelogenous leukemia (AML). This leukemia has an extremely poor prognosis; most of the victims die within 1 year of diagnosis. All of the 53 patients in the study were in complete remission from AML following chemotherapy. Twenty-one continued on chemotherapy alone while the remaining 32 received both chemotherapy and immunotherapy consisting of weekly injections of irradiated leukemia cells and of BCG. (Tumor cells used in such studies are usually inactivated by irradiation or treatment with inhibitors of cell division in order to avoid the danger of giving tumor cells that are capable of dividing—and producing cancer—to human patients.) Powles found that patients undergoing immunotherapy had longer remissions and survival times than those who had chemotherapy alone. Immunotherapy did not cure the disease, however.

The immunotherapeutic strategies discussed thus far all involve active stimulation of the patient's immune response. A different way to augment his immune defenses is to transfer specific immune capacity from another individual to the patient. Lymphocytes could be recovered, for example, from a patient cured of a particular cancer and given to another patient afflicted with the same disease. This approach entails major problems. The immune system of the recipient would recognize the lymphocytes as foreign and destroy them. Severe hypersensitivity reactions, like those occasionally observed with BCG, might also occur. Use of whole cells may be unnecessary, however. Recent research suggests that certain products of the immune system can transfer specific immunity between in-

dividuals without themselves evoking undesirable immune responses.

The two products receiving the widest attention are "transfer factor" and "immune" RNA. Transfer factor, which was discovered by H. Sherwood Lawrence of New York University School of Medicine about 20 years ago, transfers cell-mediated immunity from one individual to another, possibly by activating the recipient's lymphocytes. The material is isolated from lymphocytes. It is a conjugate of nucleic acid (probably RNA) and polypeptide. Since transfer factor has a molecular weight of less than 10,000, it is not immunogenic.

Because of these properties, investigators think that transfer factor may be useful for cancer therapy. It is especially important that it transfers cell-mediated, but not humoral, immunity. "Blocking factor" (*Science*, 3 May, p. 552), which can prevent the attack of lymphocytes on tumor cells, may be a complex of antibody with tumor antigens. Stimulation of humoral immunity—and antibody production—could increase the blocking activity in a cancer patient's serum and thus interfere with his response to immunotherapy or even enhance tumor growth. This is a potential problem with any therapy in which the immune system is stimulated, but it should not be a deterrent to the use of transfer factor.

Although many investigators think that transfer factor is specific—eliciting immune responses in the recipient only to those antigens to which the donor can respond—this has not yet been definitively established and is a matter of some dispute. Nevertheless, transfer factor for cancer therapy is often prepared from the lymphocytes of people who can be shown to have immunity to antigens associated with the tumor in question. Since evidence indicates that tumors of the same type have common antigens, the donors could be individuals cured of the particular cancer. They could also be members of the patient's immediate family or very close associates.

Morton has shown that relatives of patients suffering from osteogenic sarcoma have a much higher incidence of antibodies to the tumor than do members of the general population. Alan Levin, working with H. Hugh Fudenberg at the University of California Medical Center in San Francisco, has shown that more than 20 percent of the household contacts of individuals with osteogenic sarcoma have strong cell-mediated immunity to the tumor. These results imply—but do not prove—that a virus causes this cancer. Only a few of those exposed to the putative virus get the disease, presumably because of their failure to mount an effective immune response.

Transfer Factor and Osteogenic Sarcoma

The prognosis of osteogenic sarcoma, a bone cancer affecting children primarily, is dismal; approximately 80 percent of the victims develop metastases in the lungs within 6 months of diagnosis even though the primary lesion is removed surgically. Levin and Fudenberg have so far used transfer factor in the treatment of 12 patients with osteogenic sarcoma. Two have died and two have switched to chemotherapy, but the remaining eight have been tumor-free and without metastases for up to 18 months.

According to Levin and Fudenberg, treatment with transfer factor is not suitable for patients who already have more than one pulmonary metastasis. It causes inflammation of those lesions that may itself threaten the patient's life. As with other immunotherapies, transfer factor should be most valuable for killing residual tumor cells and preventing metastasis after successful removal of the primary tumor. Another problem is the possible depletion of the donor's capacity to mount an immune response to tumor antigens. Fudenberg has evidence that this happens after extensive removal of the donor's lymphocytes. The donors recovered the capacity after the lymphocyte collections were halted, but this finding raises the question of whether it is safe to deprive humans—especially cured cancer patients—of their immune defenses against cancer. Despite these problems, results from Fudenberg's laboratory and from a number of others are encouraging investigators to undertake more extensive, controlled experiments with transfer factor for cancer therapy.

"Immune" RNA is another substance that can be used to transfer cell-mediated immunity between individuals, according to Yosef Pilch of the University of California School of Medicine, Los Angeles. Pilch has shown that "immune" RNA, which is extracted from lymphoid tissues, can elicit immune responses against tumors both in vivo and in vitro. The effects were specific for the type of tumor used to immunize the donors of "immune" RNA but they were not species-specific. For example, "immune" RNA extracted from the lymphoid organs of guinea pigs that had been immunized against a rat tumor protected rats against the growth of transplants of the tumor.

Pilch has extracted "immune" RNA from the lymphocytes of humans cured of melanoma. The RNA could convert normal human lymphocytes to cells cytotoxic to cultured melanoma cells. Moreover, "immune" RNA extracted from lymphoid cells of sheep that had been immunized to a human melanoma doubled the cytotoxic activity of the human patient's lymphocytes against his own melanoma cells in culture.

As long as "immune" RNA is free of protein contamination, it is weakly antigenic, if at all, and is well tolerated by patients. This property, in conjunction with the ability to use animal rather than human donors and the tumor specificity of "immune" RNA, makes its potential use for cancer immunotherapy an attractive prospect.

At present there is insufficient data to evaluate and compare immunotherapeutic strategies with each other and with conventional therapies. There have been promising results, especially in treating patients with accessible tumors including melanoma and other skin cancers, but only if there are no internal metastases. Other data indicate that the capacity of the immune system to control tumor growth is limited, and many investigators think that immunotherapy must be restricted to patients whose tumor burden has already been reduced to very low levels by conventional techniqes.

There are unaswered questions and unsolved problems concerning potential hazards of immunotherapy. These include hypersensitivity reactions and the possibility of evoking an autoimmune response in which the patient's immune system attacks his normal tissues instead of just tumor cells. This could happen if the antigens used to stimulate the immune system were found on normal cells and not just on tumor cells. Stimulation of the immune system might also result in increased production of blocking factors and enhanced tumor growth. Investigators think that intensive monitoring of the patient's immune responses with in vivo and in vitro assays is required for assessing the efficacy of immunotherapies.

Other therapies in addition to those described here are being considered. Only time—and careful, well-controlled clinical trials—will tell which are most satisfactory for cancer therapy.

—JEAN L. MARX