Researcher Describes Early Success with Macrophages in Treating Metastasis

( Editor's Note: Isaiah J. Fidler, DVM, PhD, Chairman, Department of Cell Biology, is the recipient of the 33rd Ernst W. Bertner Memorial Award, presented for his work with metastatic cancer at the 1983 Annual Symposium on Fundamental Cancer Research, sponsored by UT MDAH. He has recently come to UT MDAH from the National Cancer Institute's Frederick Cancer Research Facility in Frederick, Maryland, where he was director of the Cancer Metastasis and Treatment Laboratory. He presently holds the Olla S. Stribling Chair at UT MDAH. Here he answers questions about the metastatic process and new methods for its control.)

Is metastasis the major problem in the treatment of cancer patients?

It is a major problem in the treatment of patients with solid tumors. The major cause of death from solid tumors can be attributed to metastasis, not to the primary tumor.

What is the process of metastasis? How do cells metastasize?

How cells metastasize is now beginning to be clear. In order to metastasize, cells from the primary tumor must invade the surrounding tissue. Of those cells, some pass into the circulation, either through the lymphatic system or small blood vessels. As they enter the circulation, these tumor cells are exposed to an enormous number of host factors or host cells, such as lymphocytes, natural-killer cells, monocytes, polymorphonuclear cells, platelets, antibodies, and hormones, that destroy the majority of tumor cells. The cells that survive in the circulation bind to small capillaries in distant organs, like the lung or the brain, invade the capillary wall, and enter the organ tissue where they begin to multiply.

My early work involved the investigation of the precise number of cells that survive to yield a metastasis. We injected mice with radiolabeled melanoma cells. By monitoring those radioactive tumor cells, we found that of all the cells that entered the circulation, less than 0.1% survived to yield metastasis. Other results showed that 1 g of tissue of a breast tumor in a rat would lead to the shedding into the circulation of $2 \times 10^6$ cells each day. And yet metastasis did not occur that frequently.

Can one assume then that a tumor cell's ability to metastasize is governed by chance?

Not necessarily. The estimate that so few tumor cells can complete the process of metastasis led to the following question: Can any cell in a tumor survive the steps of metastasis—invasion into the circulation, survival in the circulation, proper interaction with the host cells, getting out of the circulation, and growth—or are tumor cells heterogeneous, unequal, insofar as metastatic properties are concerned? Do very few cells in a tumor have the probability of forming metastases?

We have investigated this issue by performing several experiments. In one such experiment, a primary tumor was cultured and split. One subculture, maintained as a mass culture, was injected directly into mice and produced a uniform number of metastases in each mouse. Clonal lines were begun from cells in the other subculture; each clone when injected into a mouse, produced a different number of lung metastases, exhibiting low, intermediate, or high metastatic potential. Other experiments have confirmed these findings, indicating that some primary tumors are indeed comprised of cells with diverse metastatic capacities.

How recent is the theory of tumor heterogeneity for metastatic properties?

The original observation was recorded in 1889 by an English physician, Paget. Paget examined autopsy reports of 735 women with breast cancer. He discovered a very peculiar thing: that breast cancer metastasis occurs with predictability in some organs but not in others. He then asked whether metastasis occurs by chance or as a selective event. His conclusion was that metastasis occurs as a consequence of two forces: "the seed and the soil." Some tumor cells are better "seeds" than others, and some organs are better "soils" than others. But it takes the
Home Hyperalimentation Promotes Self-Care

To date, 32 cancer patients at UT MDAH have administered their own intravenous hyperalimentation (IVH) at home. Before 1981, when the home IVH program was instituted at UT MDAH, hospitalization was required for all patients receiving this form of nutritional support.

According to the program's coordinator, David M. Ota, MD, Department of Surgery, the program was instituted to free selected patients from the expense and mental stress of hospitalization. Many patients hospitalized to receive IVH, for malnutrition caused by cancer or by treatment, are ambulatory and well enough for short-term self-care. For this reason, a team of specialists, including a physician, nurses, a pharmacist, and a dietician, developed the program, establishing criteria for patient selection and procedures for training the patient and a family member in home IVH care.

Selection criteria were carefully determined so that those patients most likely to benefit from home IVH would be chosen. Candidates for the program must require a minimum of 6 weeks of IVH and be ambulatory, have a stable metabolic course but inadequate gastrointestinal tract function, have cancer potentially responsive to treatment, and have a family member to help with the patient's home care.

Selected patients are entered into a 10- to 15-hour course to learn the principles of good nutrition, operation and proper care of the equipment, and methods of monitoring the body's condition. The course covers the nutritional needs of each patient, considering his or her specific disease and treatment, and explains how IVH meets these needs. The patients are taught how to hang the solution bag on the movable IVH stand and adjust the infusion pump to compensate for too slow or too fast infusion, as well as the aseptic techniques used when changing dressings and cleaning the entry site of the catheter. Patients requiring continuous infusion learn to assemble and adjust equipment in a portable vest that allows them freedom of movement while receiving IVH. The patients also learn to monitor the body's metabolism by checking body weight and temperature daily and by testing the urine twice a day to measure the presence of ketones and sugar. These results are then recorded in the home IVH manual, which serves as a text for the training course. Upon completion of the course, patients are given an exit interview by home IVH team members to confirm their capability of self-care at home.

Before the patient returns home, arrangements are made for delivery of supplies and periodic patient checkup. A private company is contacted to deliver to the patient's home the individual nutrient solutions and all equipment, including the storage refrigerator and sterile dressings and tape. In addition, the patient's family physician is notified and is requested to send the results of regular blood tests to UT MDAH. The physician is also given the patient's IVH solution formula and is sent a copy of the home IVH manual for reference.

Although the program offers only a temporary life-support system, it has enabled patients to continue receiving much needed treatment without the necessity of hospitalization. A total of 2,300 hospital days has been saved in the program's first year alone.

(Physicians desiring additional information or a copy of the patient teaching manual should write or call David M. Ota, MD, Department of Surgery, MDAH Box 106, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, 6723 Bertner Avenue, Houston, Texas 77030. Made possible by a gift from Mrs Harry C. Wiess.)
CNS Irradiation of Children Found to Impair Arithmetic-Related Abilities

by Donna R. Copeland, PhD, Department of Pediatrics; Jack M. Fletcher, PhD; Betty Pfefferbaum-Levine, MD, Department of Pediatrics; Norman Jaffe, MD, Department of Pediatrics; Hubert L. Ried, MD, Department of Pediatrics; and Moshe H. Maor, MD, Department of Clinical Radiotherapy

Recent studies have reported abnormalities in the neuropsychologic functioning of children who have received central nervous system (CNS) treatment for cancer. There have been reports, for example, that CNS treatment in children may result in long-term cognitive sequelae, particularly when the treatment involves radiation. However, the studies to date provide a limited assessment of this possibility due to small numbers of study participants, inadequate or no control groups, variability in age of patients at diagnosis, and limitations in neuropsychologic test batteries.

Our study, designed to correct these deficiencies, has clarified issues raised by such investigations concerning the long-term effects of CNS irradiation and of CNS chemotherapy on children. We have found that CNS irradiation of children affects visual-motor and motor skills and spatial-processing tasks related to arithmetic, regardless of age at diagnosis; yet CNS chemotherapy alone does not appear to significantly affect cognitive functioning in children (Table 1).

Population

The study, conducted between 1980 and 1982, was comprised of 74 patients; thirty-six were female and 38 were male. The mean age at evaluation for the total group was 14 years. The mean age at disease diagnosis was 5.5 years. All patients were long-term survivors of childhood cancer. Long-term survival was defined as a disease-free state at least 5 years beyond initial treatment for cancer or for 5 years beyond the last known disease-activity.

The patients were divided into three groups according to type of treatment received. Two of these groups were comprised of survivors of childhood leukemia or lymphoma who had received required CNS prophylaxis. Group I (24 patients) had had acute leukemia or poorly or undifferentiated lymphoma, which was treated with chemotherapy only, including intrathecal (IT) medication; those in group II (25 patients) had had acute lymphocytic leukemia or acute granulocytic leukemia treated with chemotherapy, including IT medication, and CNS irradiation (approximately 2400 rad over a 16-day period). All patients in groups I and II had been free of CNS involvement at the time of diagnosis, except a patient in group II with acute granulocytic leukemia.

Group III (25 patients), a control group, was comprised of patients who had had solid tumors or Hodgkin's disease treated with surgical excision or chemotherapy or both, and, in some cases, irradiation to sites other than the CNS. Diagnoses in this group were: malignant bone tumors (7 patients), neuroblastoma (5 patients), Wilms' tumor (4 patients), soft tissue tumors (3 patients), other solid tumors (4 patients), and Hodgkin's disease (2 patients).

Patients in each group were evenly distributed in terms of sex, ethnicity, and age at diagnosis. Statistical comparisons across demographic variables revealed significant differences only between groups II and III for age at evaluation of CNS impairment; patients in group III were older than those in group II (P<.01) when tested. Because of this age difference, all dependent measures in the neuropsychologic test battery were standardized using age-based norms.

Methods

All patients were administered a battery of neuropsychologic tests that measure functional skills frequently impaired in children with neurologic disorders. Tests in this battery were based on a

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developmental model for testing children and have been applied to many different patient groups, including children with learning problems, meningitis, head trauma, and other similar problems.

The functional abilities measured and the specific tests employed are as follows: intellectual functioning: The Wechsler Intelligence Scale for children and the Wechsler-Adult Intelligence Scale measure verbal IQ (VIQ), performance IQ (PIQ), and full-scale IQ (FSIQ); visual motor and constructional skills: the Beery Developmental Test of Visual-Motor Integration and the Recognition-Discrimination Test measure hand and eye coordination; tactile-spatial skills: the Tactile Perception Test measures one's ability to identify shapes by touch; fine motor skills: finger-tapping and trailmaking tasks from the Halstead-Reitan Neuropsychologic Test battery and the Grooved Pegboard Test include such tasks as matching two dots or fitting pegs into holes; memory and learning: the Verbal Selective Reminding Test and the Nonverbal Selective Reminding Test permit separate estimates of storage and retrieval skills; language skills: the Peabody Picture Vocabulary Test, Rapid Automatized Naming Test, and Word Fluency Test measure a child's ability to express and receive information; school achievement: spelling and arithmetic subtests of the Wide Range Achievement Test and the reading recognition and reading comprehension subtests from the Peabody Individual Achievement Test measure reading and arithmetic skills.

Thirty-five age-adjusted dependent variables, scores of the various tests, were divided into nine subsets: FSIQ, VIQ, PIQ, full-scale IQ (FSIQ); visual motor and constructional skills: the Beery Developmental Test of Visual-Motor Integration and the Recognition-Discrimination Test measure hand and eye coordination; tactile-spatial skills: the Tactile Perception Test measures one's ability to identify shapes by touch; fine motor skills: finger-tapping and trailmaking tasks from the Halstead-Reitan Neuropsychologic Test battery and the Grooved Pegboard Test include such tasks as matching two dots or fitting pegs into holes; memory and learning: the Verbal Selective Reminding Test and the Nonverbal Selective Reminding Test permit separate estimates of storage and retrieval skills; language skills: the Peabody Picture Vocabulary Test, Rapid Automatized Naming Test, and Word Fluency Test measure a child's ability to express and receive information; school achievement: spelling and arithmetic subtests of the Wide Range Achievement Test and the reading recognition and reading comprehension subtests from the Peabody Individual Achievement Test measure reading and arithmetic skills.

Statistical analyses determined that the irradiated group generally performed significantly lower than the nonirradiated groups (IQ, academic achievement, and tactile-spatial subsets). Multivariate analysis of variance (MANOVA) was used to compare differences among the three treatment groups on each of these subsets. Univariate analysis of variance (ANOVA) and Tukey's pairwise procedure were used for group comparisons of the 35 variables. The MANOVA and ANOVA were also used to assess the effects of age at diagnosis.

Results

Although group I did not differ significantly from group II on as many measures as did group III, there were consistent trends in this direction (Figures 1, 2, and 3); thus, the pattern of differences between the CNS-irradiated group and each of the nonirradiated groups is essentially the same.

Significant differences in age-based mean scores between the two age groups occurred for four subsets: academic achievement (P < .013), visual-motor skills (P < .023), memory (spatial) (P < .021), and motor skills (P < .052). Poorer performance on these four subsets was associated with earlier diagnosis, except for performance on academic achievement. The children diagnosed earlier had higher reading achievement scores. It is likely that children in the older group were in treatment and missed school when reading was being taught. The majority of younger children had completed treatment for cancer before starting school. The above findings for the effects of age at diagnosis were present in all treatment groups. Irradiated children were no more affected by age at diagnosis than were children in the nonirradiated groups.

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These results show that the effects of treatment do not depend on the age of the child. Radiation was found to have a detrimental effect on neuropsychologic functioning regardless of age at diagnosis. Children irradiated at an earlier age consistently scored lower than those in the nonirradiated groups on the same subsets as did children irradiated at a later age (IQ, academic achievement, memory [spatial], and motor skills). Also, IQ scores were similarly low for irradiated children of both age groups.

Fig. 1. Performance of three treatment groups on IQ and academic achievement tests. All test performances are expressed in standard scores with a mean of 100 and a standard deviation of 15. Abbreviations are as follows: FSIQ = full-scale IQ; VIQ = verbal IQ; PIQ = performance IQ; SP = spelling; AR = arithmetic; RR = reading recognition; RC = reading comprehension.
Conclusion

Other studies have suggested that CNS prophylaxis impairs the cognitive functioning of long-term survivors of childhood leukemia and lymphoma. However, these studies have yielded a variety of conflicting results in terms of the type of treatment necessary to produce these effects and the nature and extent of post-treatment cognitive difficulties. Our study, because of (1) the number of patients evaluated, (2) use of a control group, (3) comprehensiveness and consistency of neuropsychologic testing instruments utilized, (4) rigorous statistical techniques utilized, (5) comparability across groups for sex, age at diagnosis, and ethnicity, and (6) constancy of institution and examiner, helps clarify results of previous studies pertaining to the long-term effects of CNS prophylaxis.

Results of our study are similar to those of Rowland et al.,* who reported the use of an extensive neuropsychologic test battery on large numbers of childhood cancer patients. Rowland et al. compared three groups of survivors of acute lymphocytic leukemia. In that study, a group of CNS-irradiated children, who also received IT methotrexate (MTX), scored significantly lower on IQ and school achievement tests than two other groups of children receiving MTX alone without CNS irradiation (IT-MTX and IT-MTX + IV-MTX). Because many of these children were evaluated only 1 year after CNS prophylaxis, the investigators were uncertain about the stability of the group differences over time. Our results help clarify that issue; the mean number of years from diagnosis to evaluation for CNS impairment for our leukemia and lymphoma groups was 7 to 8 years, and for the control group, 10 years. Together, these two studies imply that the effects of radiation on the neuropsychologic functioning of childhood cancer patients do not change over time. These effects seem to appear shortly after treatment (1 to 3 years) and remain in long-term survivors.


Fig. 2. Performance of three treatment groups on IQ subtests and visual-motor, motor, and spatial-memory tests. All test performances are expressed in standard scores with a mean of 10 and a standard deviation of 3. Abbreviations are as follows: INF = information; SIM = similarities; AR = arithmetic; COM = comprehension; DS = digit span; PC = picture completion; BD = block design; OA = object assembly; COD = coding; VMI = visual-motor integration; RD = recognition discrimination; LTS = long-term storage; CR = continuous retrieval; FT = finger tapping; TA = trailmaking A; TB = trailmaking B; GP = grooved pegboard; DH = dominant hand; NDH = nondominant hand.

Fig. 3. Performance of three treatment groups on language, verbal memory, and stereognosis. All test performances are expressed in standard scores with a mean of 10 and a standard deviation of 3. Abbreviations are as follows: PPVT = Peabody Picture Vocabulary Test; RAN = rapid naming; WF = word fluency; LTS = long-term storage; CR = continuous retrieval; ST-DC = stereognosis, dominant hand, correct response; ST-DT = stereognosis, dominant hand, timed test; ST-NDT = stereognosis, nondominant hand, correct response; ST-NDC = stereognosis, nondominant hand, timed test.

Whether the effects of CNS irradiation on cognitive skills are diffuse or specific is a question of concern. Although there was a general lowering of scores across all variables for the irradiated group (results often interpreted as indicative of diffuse CNS impairment), differences between the irradiated and nonirradiated groups were significant only for nonlanguage skills often associated with problems in arithmetic. Impairment of nonlanguage skills only in irradiated patients is particularly evident on the memory tasks, which employ completely analogous tests for measuring memory skills, differing only in the types of material to be remembered (verbal or visual-spatial) (Figures 2 and 3). Group II scored significantly lower than groups I and III on the visual-spatial test only. Such results indicate that the irradiated group most likely had specific rather than diffuse impairment.

The finding that the irradiated group was no more affected by age at diagnosis than the other two treatment groups questions the hypothesis that cognitive functions are more affected by radiation in younger children. Our results show that CNS irradiation is associated with poorer performance regardless of age at diagnosis.

Because the two CNS-treated groups (groups I and II) received similar chemotherapy and had equal opportunity for school attendance, CNS irradiation (or possibly CNS irradiation combined with IT chemotherapy) appears to be the primary cause of abnormalities in the neuropsychologic functioning of group II children. This conclusion is supported by the lack of difference between scores of the nonirradiated groups I and III.

These findings suggest that in the absence of irradiation, CNS treatment does not have a measurable effect on the neuropsychologic functioning of children. Results of this investigation should support the trend to employ chemotherapy alone for children with cancers, such as leukemia and lymphoma, that require CNS prophylaxis.

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Metastasis . . .
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right "seed" and the right "soil" to produce a metastasis. Paget said almost 100 years ago that in this biologic process we must consider the tumor cell properties and host factors, which together determine the outcome of the interaction. What I've been saying is simply a reinvention of Paget's "seed and soil" hypothesis. A tumor with some cells better able to metastasize than others exhibits diversity, not uniformity. The scientific term for diversity is heterogeneity.

Was Paget's theory widely accepted, or forgotten?
The idea was debated and discarded. A more popular hypothesis originated in the middle 1920s: that metastasis occurs most frequently in the lungs and the liver because of hemodynamics. Tumor cells of abdominal tumors were thought to travel through the circulation to the capillary bed of the liver. Tumor cells of tumors growing on the body's periphery, the breast or skin for example, were thought to metastasize to the lung by way of the venous circulation where the blood drains first to the heart and then to the lung.

Very detailed experiments have been performed in mice to test this hypothesis. In one such experiment in our laboratory, a small fragment of lung of a mouse was embedded in a muscle of one leg, and a small fragment of kidney was implanted in the other leg as a control. The mouse was then injected with melanoma cells exhibiting preferential growth in the lung. If the tumor were to metastasize to the lung because its capillary bed was first encountered by the tumor cells, then metastasis would occur only in the natural lung, not in the lung fragment implanted in the leg muscle. If metastasis were to occur as a consequence of interaction between the tumor, "the seed," and the organ, "the soil," metastasis would grow in the natural lung and in the implanted lung fragment in the leg, but not in the implanted kidney in the other leg. And that's exactly what happened. The melanoma proliferated in the natural lung (the in situ lung) and in the implanted lung fragment, but not in the implanted kidney.

Most researchers today agree that metastasis is not a chance event and that tumor cells are indeed heterogeneous for metastatic properties.

Why have we not been able to control metastasis? Is it because of tumor heterogeneity?
Partially. The primary reason is that in the majority of patients, except those with skin cancer, by the time of diagnosis, metastasis has already occurred. If there are already tumor colonies in the liver, for example, removing the primary tumor has little effect on the proliferating cells in the liver. Also, because the metastasis may be comprised of cells different than those in the primary tumor, the metastasis may be unresponsive to drug therapy designed for the primary tumor. In addition, cells in one metastasis may be distinctly different from cells in another metastasis. This fact is often evident clinically. If a patient presents with multiple metastases, sometimes several metastases regress while one progresses during chemotherapy.

Why have you chosen macrophages to help combat metastatic cancer, rather than T cells, natural-killer cells, or any other defense cell?
Because the major dilemma for the treatment of metastasis is that metastatic tumor cells are biologically very diverse. I was therefore intrigued by the observations, made many years ago in my own lab and others, that activated macrophages harvested from animals with infection can kill tumor cells, but not normal cells. Moreover, activated macrophages in culture kill tumor cells whether or not these cells are sensitive or resistant to lymphocytes or natural-killer cells or sensitive or resistant to drugs. T cells kill tumor cells, but we can select for tumor cells resistant to T cells. We can select for cells resistant to natural-killer cells. But no one has been able to select for cells resistant to macrophages. I'm not talking about increased sensitivity or decreased sensitivity to macrophages. I'm talking about absolute resistance. We have not detected it, nor has anyone else.

So we realized the following: The major dilemma in cancer treatment is to eliminate those cells that conventional treatment cannot eliminate. With a 1-cm tumor, or approximately $1 \times 10^9$ cells, the destruction of 99.9% leaves $1 \times 10^6$ cells to proliferate. These are the fatal tumor cells. Therefore, the challenge to the basic researcher interested in therapy is not to design more and more drugs that can eliminate the 99.9% of tumor cells. The challenge is to design an approach that will destroy the remaining...
Activated human blood macrophages lyse a human melanoma. Macrophages were activated with liposome-encapsulated muramyl dipeptide. (This scanning electron micrograph of activated macrophages, produced in Dr. Fidler’s Laboratory at the National Cancer Institute’s Frederick Cancer Research Facility, was provided by Dr. Corazon Bucana.)

small number of fatal cells. We believe that macrophages can accomplish this task.

Must macrophages be activated to be effective against tumor cells? If so, what method do you use?

Yes, macrophages normally operate in the body at low levels and must be activated to lyse tumor cells. In vivo, there are two major ways in which macrophages are activated: by interaction of macrophages with micro-organisms, such as bacteria and its products, and by interaction with lymphokines.

The first method of activation occurs in every multicellular animal. Macrophages in the horseshoe crab, a living fossil, exhibit panic reactions to endotoxins in the same manner as human macrophages. Macrophages are also activated in vivo by lymphokines, soluble mediators released by T cells when stimulated by an antigen.

It is very difficult to activate macrophages in vivo by systemic injection of lymphokines or a bacterial product because of the side effects. To deliver these activating agents to macrophages, we use synthetic membranes called liposomes or lipid vesicles made of phospholipids, basic components of every cell membrane in the body. By shaking these synthetic membranes in a test tube we create a multilaminal, multilayered ball that, when cut crosswise, looks like an onion. The liposome is used to carry the activating substance, which remains trapped between the membrane layers.

The liposomes are injected into animals intravenously. Why? A major role of macrophages in the body is to clear dead cells, dead bacteria, and foreign particles from the bloodstream. Liposomes injected intravenously are phagocytosed by macrophages; if the liposomes contain activation signals (a bacterial product or lymphokines), the macrophages then respond to these stimuli.

The bacterial product we use is muramyl dipeptide (MDP), a synthetic molecule identified as the basic unit in a bacterial cell wall that leads to immune potentiation. Experiments with other liposome-encapsulated substances are ongoing. Most interesting are the results obtained by packaging in the same liposome two different signals, such as MDP and lymphokines; synergism occurs. Superior results in macrophage activation and treatment of metastasis are achieved when each liposome contains two diverse activation signals, as compared to results achieved with a single liposome-encapsulated substance.

How have you tested the effectiveness of macrophage activation in controlling metastasis? What were the results?

The routine experiment has been to inject mice intradermally with melanoma cells and allow lung and lymph node metastases to develop. After the primary tumors are surgically removed, the mice are treated by repeated injections (2 injections a week for 4 weeks) of liposomes that contain an immunomodulator or a combination of substances; control mice are treated with liposomes that contain placebos. The survival of the mice is then recorded. In our laboratory, control mice died within 90 days after the experiment began. Of those mice receiving macrophage therapy, 60 to 70% survived until the experiment was terminated (approximately 1 year after it began). Survival was associated with measurable regression of metastases. Other researchers have had similar results.

How do you know such results occur from macrophage activation rather than from the activity of other defense cells?

Several experiments indicate that macrophage activation produces these results. First, if we depress the macrophage system in a mouse, liposome treatment fails. Second, we can harvest macrophages that are very cytotoxic from regressing tumors. These results indicate that macrophages are central or essential cells. Mind you, I’m not saying that macrophages are the only active cells; they may indeed recruit other cells. However, we have obtained therapeutic results using our method in nude mice, which are totally deficient in T cells, and in mice whose natural-killer cells have been depleted.

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Metastasis . . .
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How will your method be used most effectively in the treatment of cancer patients?

A multifactorial disease such as cancer must be treated by multiple approaches. Tumor cell destruction by macrophages is very limited. Macrophages first must find tumor cells and then form direct contact with them. In a mouse, we estimate that macrophages can kill approximately 1 to 5 x 10^6 cells and no more. This is a very small number of tumor cells.

To overcome this limitation, we are investigating a number of multimodal approaches. The first is to use macrophage activation after the bulk tumor has been removed by surgery or after radiotherapy. The second approach is to use macrophage activation in combination with chemotherapy.

Chemotherapy used alone is limited by host toxicity. When chemotherapy is used with macrophage therapy a smaller drug dose is required, thus alleviating the problem of host toxicity. Toxicity can then be defined as the quantity of the drug that will interfere with macrophage activation. For Adriamycin, for example, we have found that dose to be approximately 2.5 mg/kg of body weight in mice. We have given this dose in mice (the usual dose is 13 to 15 mg/kg) and then a week later have activated macrophages with liposome-encapsulated substances. The logic is that if we administer a small drug dose to reduce the tumor burden from 10^9 to just 10^6 cells, for example, macrophages can then be activated to destroy what remains. We give optimal chemotherapy and maximal macrophage activation. Thus, both modalities are used for maximal benefit.

When do you think we will start using these methods in patients?

I don't know. Toxicity studies are required. Macrophage activation is not toxic in mice and guinea pigs, and that's encouraging. These results, however, cannot be immediately translated to humans. Muramyl dipeptide toxicity studies have never been carried out in humans.

Why have you come to UT MDAH? What do you hope to accomplish?

My work at UT MDAH will allow me to test in clinical situations many hypotheses developed in mice and in experimental animals. The interaction between us and our clinical colleagues at UT MDAH has been outstanding.

If you do see this method used successfully in humans, what areas do you hope to explore next?

Our research continually raises other questions. We know a great deal about how metastasis occurs. We don't know why it happens. All things in nature occur for a reason. I would like to understand the reason cancers metastasize.

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