

The Immune System

The immune system is the body's defense system. It works on three different levels. The first level is the anatomic response. It consists of anatomical barriers to foreign particles and includes the skin and acid in the stomach. Anatomic barriers prevent foreign substances from entering the body. If foreign particles pass through the first line of defense the second line of defense called the inflammatory response kicks in. The third line of defense is the immune response. It is the main player in specific immune defense.

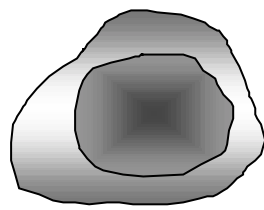
The cells of the immune system mount the immune response. These cells are also called white blood cells.

There are several types: The neutrophils are responsible for killing bacteria and yeast and are the first white blood cells at the site of an infection. The eosinophils play a part in delayed reactions to foreigners. The key players that will be discussed here are the monocytes and the lymphocytes. Monocytes are scavengers. They scour the body for anything out of place. They can engulf foreign particles and chew off pieces of tumor cells. Lymphocytes are not able to engulf any foreign particles or eat tumor cells. They take the information given to them by monocytes and monocyte-like cells and do their job. There are several types of lymphocytes. The types essential to this topic are the B lymphocytes, and the T lymphocytes. The B lymphocytes get their characteristics after being nurtured in the bone marrow, hence the B. B lymphocytes are primarily responsible for producing antibodies. Antibodies can inactivate bacteria, fungi, and viruses and make them and other foreign particles easier to see by the rest of the immune system. T lymphocytes mature in the thymus gland, which is located under the breast bone, hence the T. For the purposes of this topic they can be divided into three major categories: the T helper cells, the T suppressor cells, and the cytotoxic T cells. The T helper and suppressor cells do exactly what their names imply. The cytotoxic T cells are primarily responsible for killing virally infected, and tumor cells.

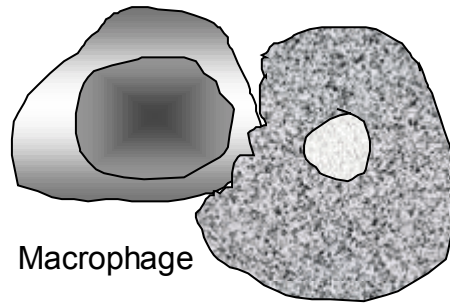
The Immune System and Cancer

In order for cancer to occur, the immune system must have failed. The normal sequence of events when the immune system comes across tumor cells follows.

An immune cell called the macrophage (also called a monocyte) comes into contact with a cancerous or precancerous cell. This cell has some strange surface features. The strange features signal the macrophage that the cell is not healthy and that the macrophage should take a bite out of it.



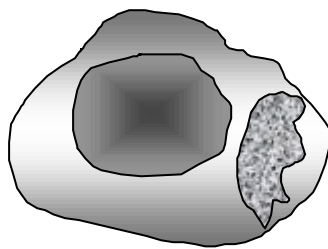
Monocyte or
macrophage



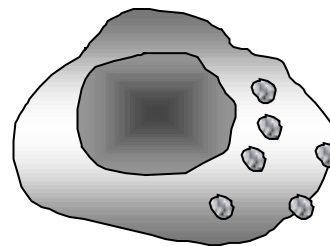
Macrophage

Tumor cell

The macrophage then begins to digest the bite of the tumor cell. Several little packets of enzymes act like a cellular stomach and break down the piece into smaller and smaller pieces.



Macrophage with
piece of cell

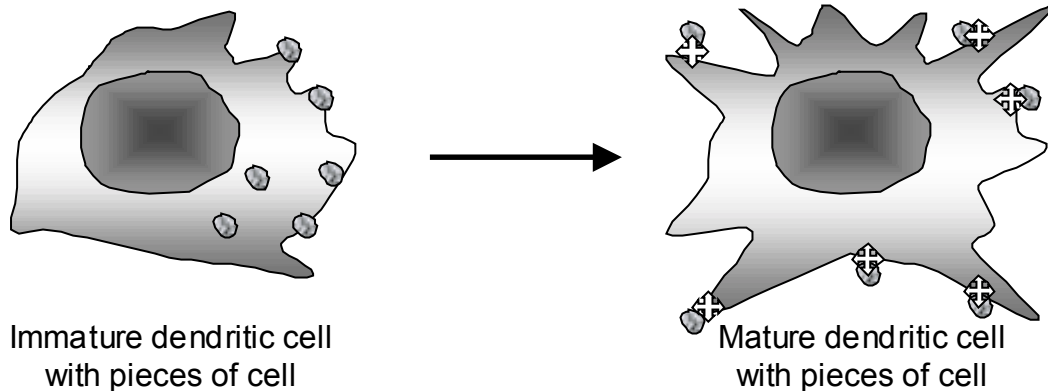


Macrophage with
pieces of cell

There are two possible scenarios that can happen next. The macrophage can either “hand off” these little pieces of tumor cell to another type of immune cell, or it can transform itself into another, specialized immune cell called a dendritic cell. There is more and more evidence that the latter happens most often.

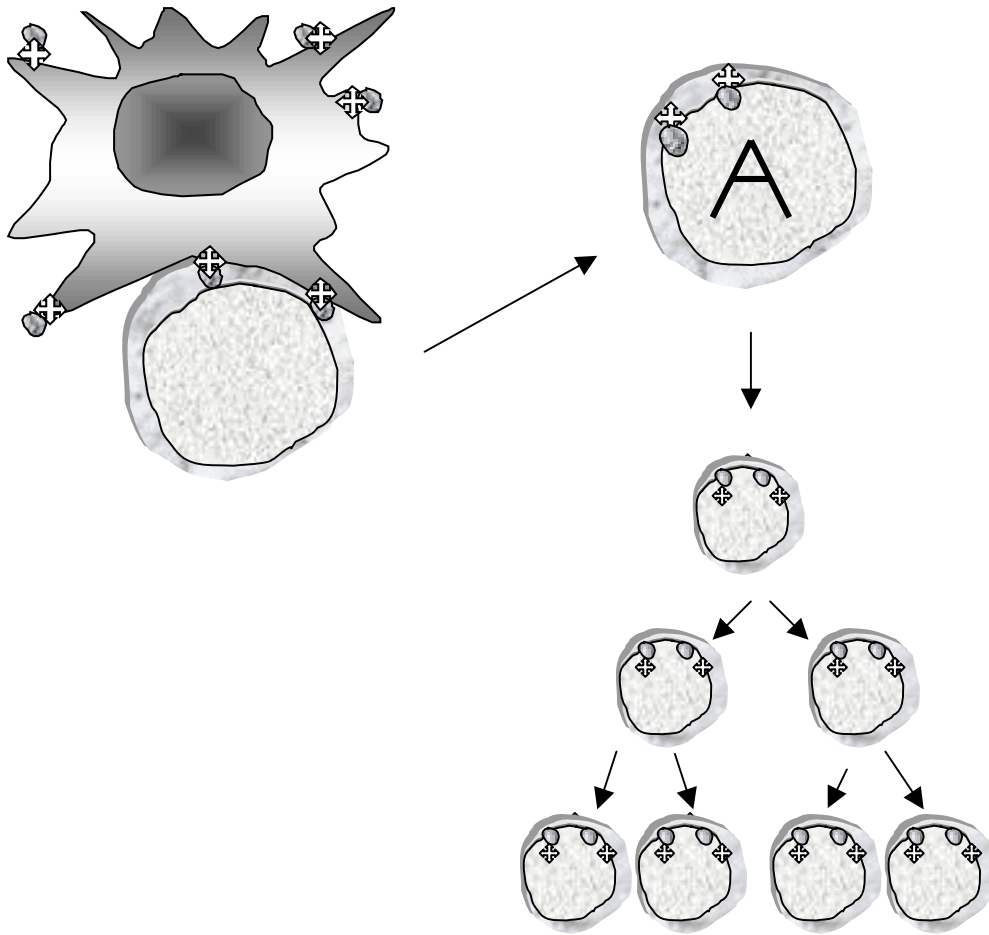
Dendritic cells are found in all tissues of the body, and many of them began as macrophages. The first dendritic cell discovered is found throughout the skin and is called a Langerhan’s cell.

Now that the macrophage has digested pieces of the tumor cell, it transforms into the dendritic cell. The dendritic cell is a much more effective messenger. When it is fully mature, it gives the information about the tumor contained in the small digested packets to the rest of the immune system. A key point here is that the dendritic cell must be mature to effectively present the tumor information. The cell needs to have additional markers on its surface that the other immune cells can recognize. These markers are called co-stimulatory molecules and are shown as white crosses on the picture of the mature dendritic cell below.

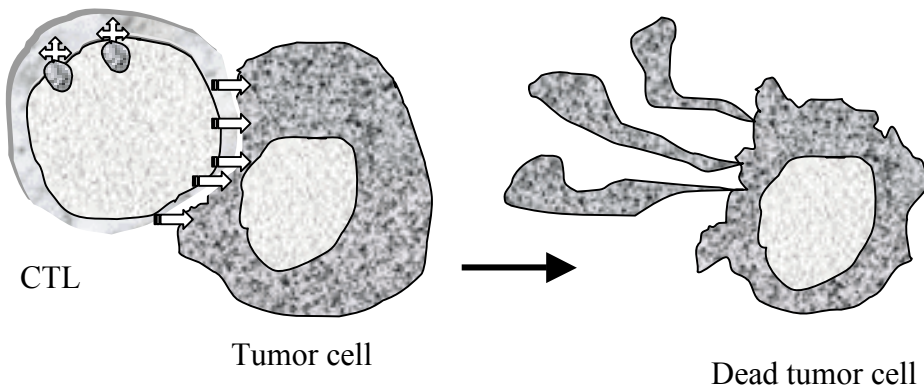


When the dendritic cell begins to mature, it also starts moving, or migrating toward a lymph node. The lymph nodes contain large numbers of lymphocytes, another type of immune cell. Everyone probably remembers having enlarged lymph nodes in their neck when they had a sore throat. The lymph nodes are where the action is when it comes to the immune system. There are areas in the body that contain large numbers of lymph nodes. The neck, armpits, and groin areas all have clusters of nodes that lie close to the skin.

So the mature dendritic cell has migrated to the lymph node. There it comes in contact with different kinds of lymphocytes. If it has matured properly, the co-stimulatory molecules on its surface will help pass the tumor information along to the cytotoxic T lymphocytes, or CTLs. The CTLs are the body's main defense against tumor cells. When the right CTL comes in contact with the dendritic cell, it will become activated and begin to divide, effectively making an army of "cloned" soldiers ready to kill any cancerous or pre-cancerous cell having the same altered membrane discovered by the macrophage.



When the CTL soldiers come in contact with cells that have the same surface as the original cancerous cell, they bind to it. They then release a chemical that pokes tiny holes in the membrane of the tumor cell, and the tumor cell spills its guts and dies.



Let's summarize what happens normally in the body after a normal cell turns cancerous. First, a macrophage comes in contact with the tumor cell, which has a different type of membrane that signals the macrophage to eat part of it. The macrophage then digests the eaten tumor cell fragment and starts to turn into a dendritic cell. It then begins to mature, and travels to a nearby lymph node and hands off the tumor cell information to CTLs. The CTLs then divide, circulate throughout the body, and kill any tumor cells they come in contact with.

Above we covered what happens normally in the body when a cell becomes cancerous. This process occurs countless times as cells get genetic mutations and become cancerous. But, if you have cancer, then something must have gone wrong. Did the macrophage fail to recognize the funny cell surface? Did macrophages not become dendritic cells? Or did the T cells not do their job? It is impossible to tell for sure—but there are some clues that the problem is with the dendritic cells.

Lately several research groups have been looking at the dendritic cells in and around tumors. What they're finding is that there are dendritic cells there, but they are immature. They don't have the co-stimulatory molecules necessary for the successful hand off of the tumor cell membrane information to the T cells. Moreover, because they are immature, they are much less likely to migrate to the lymph nodes to make the hand off.

To make a football analogy, the dendritic cell is the quarterback and needs to hand off the football to the running back (the T cell). In order to do that, he needs to move toward the running back and hand him the ball without fumbling. When the dendritic cell is immature, it just stands in one place and drops the ball. If that continues to happen, your team never scores and ultimately loses the game.

If you cut up a piece of tumor from kidney cancer or renal cell carcinoma and look at it under the microscope, you'll find millions of dendritic cells—many more than in any other type of tumor. Expectedly, the majority of these dendritic cells are immature—they don't have co-stimulatory molecules on them. What makes this more interesting is the fact that kidney cancer is the most likely type of cancer to disappear without a trace without any treatment, or spontaneously remiss. What I believe happens when someone has a spontaneous remission is the conditions in and around the tumor change enough to allow at least some of the dendritic cells to mature. This is more likely to induce a remission in renal cell carcinoma simply because of the larger numbers of dendritic cells.

So, what can you do to get dendritic cells to hand off information about your tumor cells to your CTLs? Both animal and human trials of using dendritic cells in the treatment of cancer have shown promising results and give us a direction in which to go.

Mayordomo et al.¹ inoculated mice with different types of cancer and allowed the tumors to develop for one to two weeks. Dendritic cells were isolated from the bone marrow of these mice, cultured with some growth factors, and exposed to tumor peptides (information about the tumor cell membranes). These 'primed' dendritic cells were then injected back into the tumor-bearing mice every four to seven days. Recovery, measured

as halting of tumor growth and subsequent regression, was seen 7-10 days after the first injection of dendritic cells. Using this treatment, cure rates of 80% for mice with Lewis lung carcinoma and 90% for mice with sarcoma were achieved.

In a similar study, Nair, et al.² induced malignant melanoma lung metastases (new tumors that spread from the first tumor) in mice, and then surgically removed the primary tumor. The mice were then treated with dendritic cells which had been 'primed' in a manner similar to that described above. Of the seven treated animals, four had no visible lung tumors, two had fewer than five remaining tumor nodules, and one mouse had 15 nodules. The number of nodules in control mice, those that did not receive dendritic cell therapy, were too many to count, but comprised approximately three-quarters of the lung by weight.

Hsu et al.³ at Stanford University pioneered the use of dendritic cell therapy of cancer in humans. They purified dendritic cells from the circulating blood of four patients with B cell lymphoma previously treated with chemotherapy. The dendritic cells were cultured and treated with antigen (tumor cell membrane information) derived from the patients' tumors. The dendritic cells were given using vein injections on 4 occasions; subcutaneous (under the skin) injections of the tumor antigen and a protein that helps stimulate an immune response were injected two weeks after each dendritic cell injection. All of the patients developed measurable T cell immune responses after one or two vaccinations. Meaningful clinical responses were seen. There was one partial response, one minor response, and disease stabilization in three patients with progressive measurable disease, and a complete response in a patient with minimal detectable disease. All of the patients have remained progression-free for two years.

Gerald Murphy, M.D.⁴, and his team at Northwest Hospital's Pacific Northwest Cancer Foundation have been testing the use of dendritic cells in patients with advanced prostate cancer. They cultured monocytes (macrophages) from circulating blood with growth factors and small pieces of protein found on the surface of prostate tumor cells. These dendritic cells were then reinfused into the patients through an intravenous drip. They performed two studies. More than 27% of study patients who participated in both clinical trials showed some improvement and the disease was stable in another 33%. All of the patients in the study had advanced prostate cancer and were unresponsive to conventional therapies, including hormone treatment.

In addition to lymphoma and prostate cancer, the deadly skin cancer malignant melanoma has been treated successfully using dendritic cell therapy. In a recent human study by Nestle et al.⁵, dendritic cells were used to treat sixteen patients with advanced metastatic (the cancer has spread) melanoma. Objective responses were seen in 5 of the 16 patients. There were two complete responses and three partial responses with regression of metastases in several organs, including skin, lung, and pancreas. The participants were followed for 15 months and no cases of autoimmunity—a potential side effect of the therapy—were found in any of the patients. The authors concluded, "...vaccination with autologous [derived from the person's own body] dendritic cells generated from

peripheral blood is a safe and promising approach in the treatment of metastatic melanoma.”

In the studies quoted above, there were little to no side effects. Murphy reports transient hypotension (temporary low blood pressure) as the only side effect seen in his study.

Given all of this compelling evidence that dendritic cells may hold a key position in effective, non-toxic treatments for cancer, we began studying them.

Our research to date has focused mainly on methods of:

1. Producing large numbers of dendritic cells from the circulating blood of cancer patients;
2. Finding the source of tumor material (antigen) for each type of tumor that will best stimulate the T cells to proliferate and kill tumor cells; and,
3. Stimulating the dendritic cells already in and around the tumor to mature, and become better T cell stimulators.

What we have found so far is that we can produce large numbers of dendritic cells from the circulating blood, give them tumor antigen, and mature them. These dendritic cells in culture are able to stimulate large numbers of T cells to become active against tumors. We are now setting out to determine if this is possible in humans.

In order to study the value of dendritic cells and activation of dendritic cells as anti-tumor therapies for patients with metastatic prostate cancer we are currently performing four clinical studies described below.

1. DENDRITIC CELLS TREATED WITH PATIENT’S OWN TUMOR MATERIAL

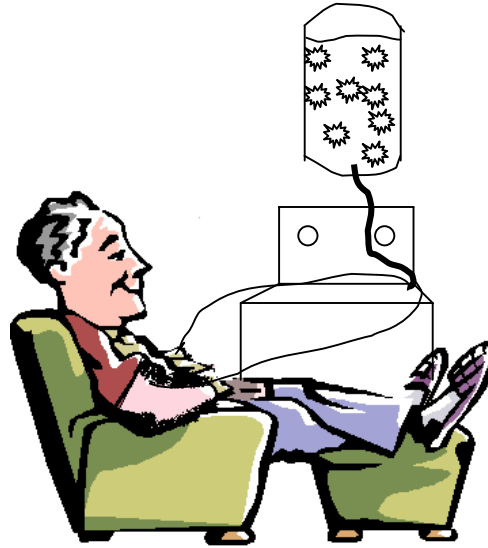
Before beginning on this protocol, patients must first provide tumor tissue to the laboratory for use with the dendritic cells. Arrangements are made with your surgeon, or urologist prior to surgery for proper collection and transport of the tumor material to the laboratory. At the laboratory, an extract of the tumor tissue will be made, and then filtered to make it sterile.

This study uses the patient’s own tumor material and own dendritic cells. Monocytes are first harvested from the circulating blood using a specialized machine. The machine is called an apheresis (Greek for “to take out”) unit. This type of machine is used at many American Red Cross offices to harvest blood products such as platelets (cell fragments that help blood to clot). The procedure is relatively simple. A needle connected to sterile, one-use tubing is placed into each arm. The tubing is connected to the machine, and approximately one cup of blood is circulated out of one arm, through the machine, and back into the other arm. The machine spins the blood, removes two types of white blood cells, and returns the fluid part of the blood and all of the other blood cells to the patient. Using this machine allows for many more white blood cells to be collected than

by simply drawing blood because the other cells are returned to the patient. The procedure takes about two hours.

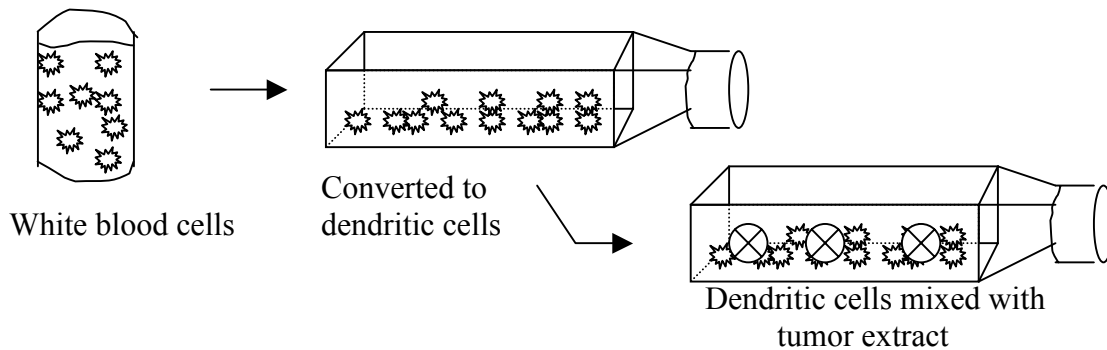


Apheresis Unit

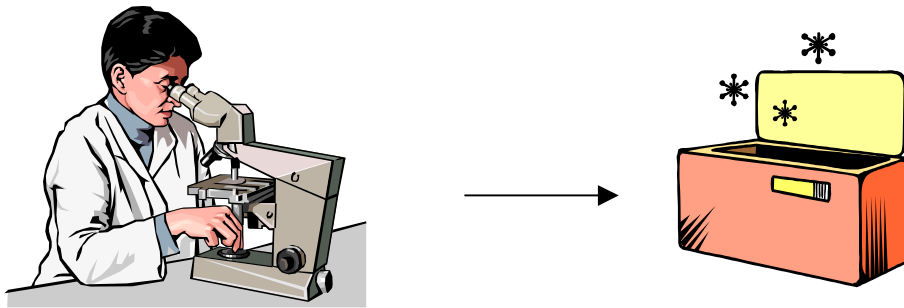


Patient connected to the machine

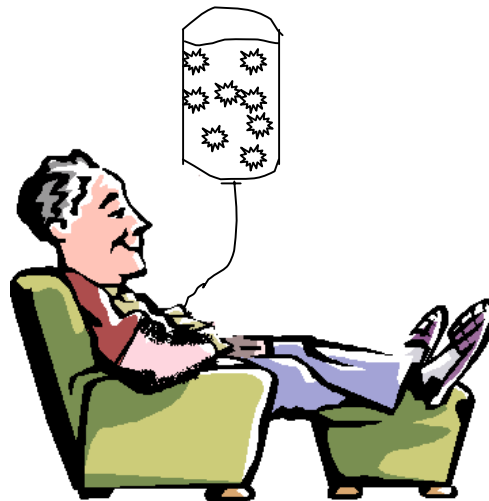
The white blood cells are then taken to the laboratory where they are grown in a special growth broth that begins to convert them to dendritic cells. After a few days the filtered tumor extract is added to the growth broth. The cells are then allowed to mature for another few days.



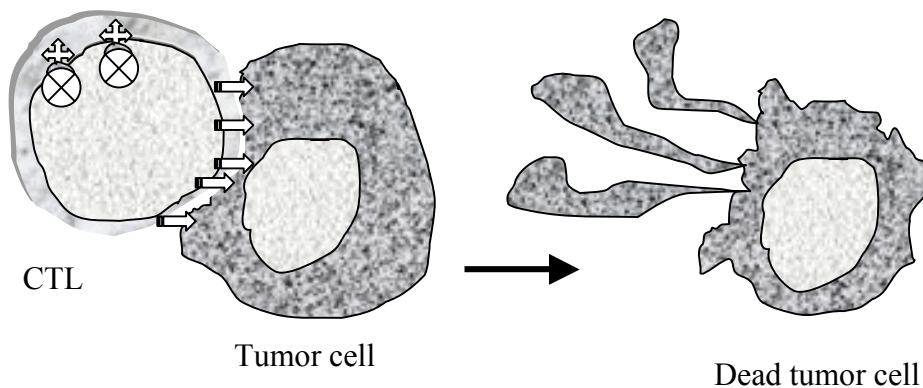
The cells are then tested for their maturity and purity. They are then frozen in liquid nitrogen and are ready to be reinfused.



A portion of the cells will then be suspended in sterile saline reinfused intravenously (by a vein in the arm) into the patient. The patient will receive an infusion once or twice per month.

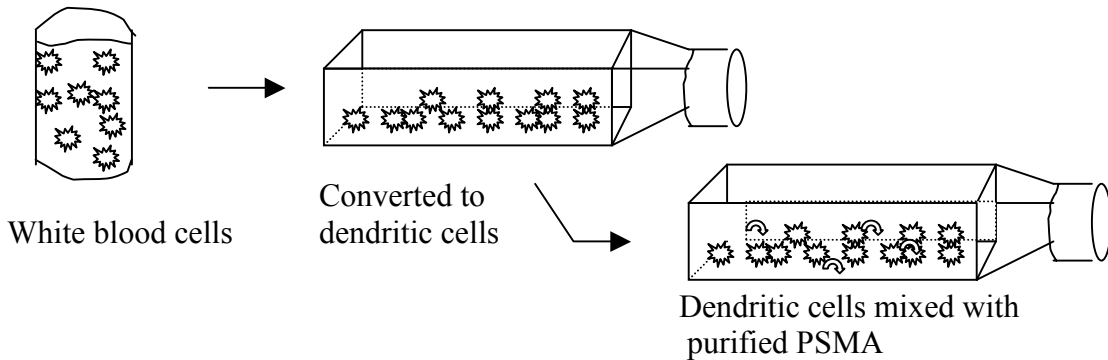


After the cells are infused, some will migrate to the lymph nodes and some will stay in the circulation. The likelihood is high that many of the dendritic cells will come in contact with T-lymphocytes (CTL's) and stimulate them to divide and recognize and kill tumor cells. Measuring the serum levels of prostate specific antigen, or PSA can allow your doctor to follow the course of prostate cancer. This test is used to determine if the treatment has been effective.

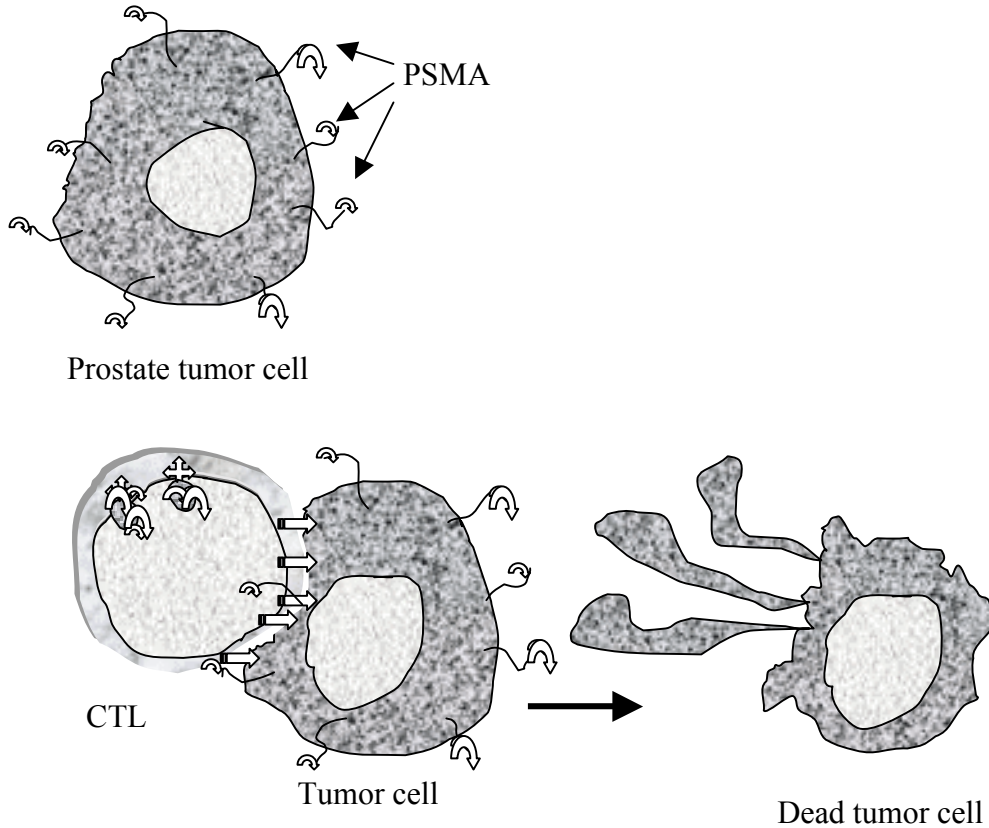


2. DENDRITIC CELLS TREATED WITH PURIFIED TUMOR ANTIGEN

This procedure is the same as number one with one exception. The agent used to prime or activate the dendritic cells is not an extract of the patient's own tumor. Instead, a known tumor antigen (molecule that has information about the outside of tumor cells) is placed with the dendritic cells while they grow and mature in culture. PSMA, or prostate specific membrane antigen, is used.



PSMA is found primarily on the outside prostate cells. The priming agents used by Murphy, described above, were fragments of the PSMA molecule.

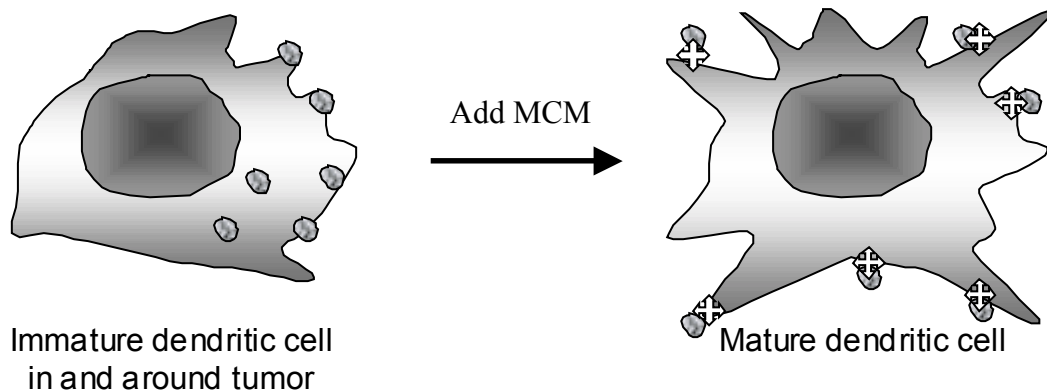


Measurement of the serum levels of PSA will also be used to determine if the treatment has been effective.

3. STIMULATION OF IMMATURE DENDRITIC CELLS TO BECOME MATURE DENDRITIC CELLS.

In many types of tumors the dendritic cells in and around the tumors are immature—meaning they are there, they just can not effectively pass along information about the tumor cells to the rest of the immune system, in particular to the CTL's, or T-lymphocytes.

One way to convert immature dendritic cells into mature ones is by using a mixture of growth factors and stimulating factors called cytokines. Many people have heard of some of these cytokines. Interleukin 2, interferon, and tumor necrosis factor are a few. These growth factors are found naturally in the body and can be manufactured using a patient's own white blood cells. The monocytes, when mixed with a medication made from bacterial cell walls, can produce an effective mixture of cytokines. The monocytes are mixed with the medicine and grown in an incubator for 2 days. Then the culture medium, or broth, is collected. It contains a lot of cytokines. We call this mixture MCM, which stands for monocyte-conditioned-medium. When MCM is placed with immature dendritic cells, a majority of them become mature. They can then migrate and effectively convey information about the tumor to lymphocytes, stimulating them to divide at the same time.

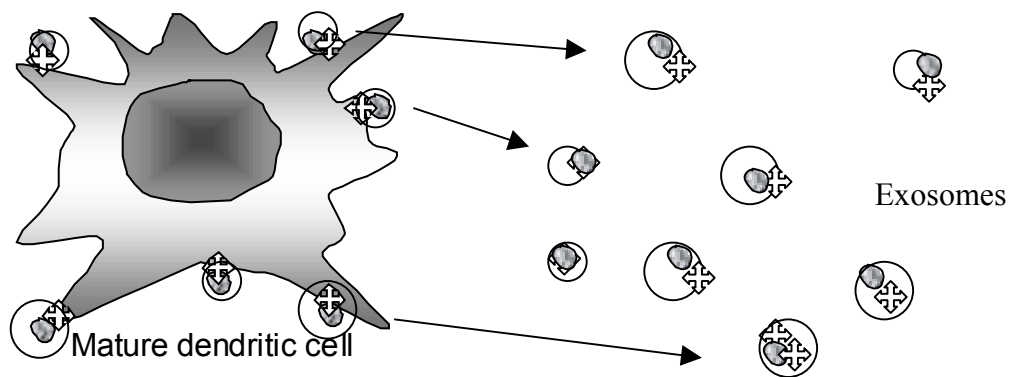


Because the MCM is prepared from the patient's own cells, the dosage varies from individual to individual. Because these cytokines are the same molecules that can make you feel poorly and give you a fever when you have the flu, the dosage is increased gradually to make sure the patient doesn't experience those side effects. The MCM is sterile filtered, and then mixed with sterile saline. It is given in the vein the same as the dendritic cells. An increased dose will be given daily until a rise in the patient's body temperature is seen. That dose will then be given daily for two weeks.

The serum PSA test is also used to follow the effects of the treatment on the course of prostate cancer.

4. CELL FRAGMENTS OF DENDRITIC CELLS PRIMED WITH TUMOR CELL ANTIGENS

Researchers in France recently discovered that when dendritic cells are grown in culture they form and release small cell fragments named exosomes. These exosomes contain all of the information necessary to activate cytotoxic T lymphocytes against tumor cells. In one study, a significant number of tumor-bearing animals had a complete remission after receiving a single injection of exosomes into the skin.



This study begins similarly to studies one and two. Monocytes are harvested from the peripheral blood. They are cultured with cytokines and tumor cell antigens are added. The cells are then allowed to mature. Instead of using the dendritic cells the liquid in which the cells are growing is collected. The exosomes are then removed from the liquid. They are sterile filtered and injected into the skin just above the lymph glands in the groin. The theory is that there they interact with the dendritic cells in the skin, which move into the lymph glands and present tumor antigen to the lymphocytes. Which then divide, circulate in the body, and kill the tumor cells they come in contact with.