THE CANCER REVOLUTION

Additional Material for Appendix 1

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Dendritic cell cancer vaccine

The most powerful anti-cancer agent is the immune system. In fact, it's impossible for cancer to develop in a body in which it is functioning correctly. The immune system defends the body against pathogens which can cause infections and disease, including cancer.[1] Pathogens are foreign bodies which can be viruses, bacteria and microorganisms.[2] The immune system's first line of defence is the skin which is the exterior barrier. The immune system also includes the cilia, which line the respiratory tract and work to expel foreign bodies; hydrochloric acid, produced by the stomach which can destroy microoganisms; intestinal flora which inhibit the growth of pathogens; the lymph nodes which secrete lymph to take pathogens away to be expelled by the body; and the white blood cells, which are produced within the bone marrow to seek, destroy and dissolve pathogens, infection and disease.

The immune system is well equipped with anti-cancer lymphocytes (white blood cells)[2] including T lymphocytes (T cells)[4] and natural killer cells (NK cells)[5], which are able to inhibit the formation of tumours by attacking and destroying mutated cells. But, the immune system can breakdown when a person is exposed to stressors which can be physical and/or emotional. Examples of physical stressors include environmental toxins, such as pesticides and household cleansers, chronic infections and radiation to name a few. Emotional stressors may include prolonged grieving, chronic fear or anxiety, and relational problems. The immune response to stressors and pathogens is inflammation. Chronic inflammation is detrimental to the body so the immune system produces T reg cells[6] to suppress the immune response to keep in in check. It is common for cancer patients' immune systems to no longer combat cancer due suppression by T reg cells or cancer treatments such as chemotherapy and radiotherapy.

The challenge

A tumour's microenvironment is capable of suppressing the anti-cancer immune response and promoting angiogenesis.[7] Herein lies the challenge— how can an anti-cancer immune response be solicited when the tumour's microenvironment, and other factors such as chemotherapy, is suppressing the immune system? Cancer researchers have proposed that a vaccine can be developed that targets cancer by encoding dendritic cells with a tumour associated cancer antigen, activating the T cells and NK cells against the cancer, and presenting these activated cells to the tumour.

A cancer vaccine: the medical holy grail

The polio vaccine has been effective in combating the polio virus. In fact, polio has been eradicated in North, Central and South America since the last case detected was in Peru in 1991.[8] Vaccines have been incredibly effective against polio as with a number of other diseases. The medical Holy Grail would be a vaccine that could eradicate cancer from the world.

A vaccine is defined as, "Any preparation used as a preventive inoculation to confer immunity against a specific disease, usually employing an innocuous form of the disease

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agent, as killed or weakened bacteria or viruses, to stimulate antibody production."[9] Traditionally, vaccines are protective, and are given to a person with the intent of preventing the contraction of a specific illness. There are a number of vaccines in use that are given as a preventative measure against only two types of cancer—cervical cancer and liver cancer. These vaccines do not directly target cancer. The two cervical cancer vaccines, Gardasil® and Cervarix®, are designed to protect against strands of the human papillomavirus (HPV).[10] The efficacy of these vaccines is debatable and more data is required to determine if the immune response against HPV is translating to a decrease in cervical cancer.[11] The liver cancer vaccine also does not targets the hepatitis B virus which is associated with the development of liver cancer. All other cancer vaccines are not protective; they are therapeutic.

Dendritic cell vaccines

Therapeutic vaccines are designed to be administered to people who already have cancer. Their purpose is to induce an immune response capable of targeting specific cancer cells, killing them, slowing or reversing tumour growth, inhibiting metastases, and ultimately increasing survival rates.[12] Most of these immunotherapy vaccines belong to the category of dendritic cell vaccines (DCV)[13], which are antigen-presenting cell vaccines.[14] The efficacy and safety of DCVs are being demonstrated in multiple clinical trials in vitro, and in vivo with animals and humans. If fact, the the FDA approved the first DCV in 2010 after phase three studies in patients with metastatic prostate cancer showed a 3-year survival rate of 34% verses 11%.[15]

Before I explain how DCVs work, let me explain what a dendritic cell is. A dendritic cell is special type of immune cell that induces an immune response by presenting antigens found on the surface of cancer cells, or infections and viruses, to immune cells that can destroy the cancer cells.[16] Dendritic cells are also referred to as antigen-presenter cells.[17] Antigens are key. Antigens could be any substance that induces an immune response.[18] Cancer cells produce antigens which are tumour associated proteins on the surface of malignant cells. Antigens also circulate in the blood. Each tumour associated antigen is unique, and that is why pathologists and oncologists use antigen counts as tumour markers to evaluate the activity of specific cancers. You are probably familiar with the term "PSA" because it is the most common test to screen for prostate cancer. PSA stands for "Prostate Specific Antigen."[19]

How DCVs work

DCs are used in vaccines because they are specialised at capturing tumour associated antigens and converting them from a protein into peptides that will attract T cells and NK cells.[20,21] They are able to induce an immune response that will kill tumour cells while retaining a memory of this response in the event that the antigen is detected again in the future.[22] It is amazing to look at how the different immune cells interact to destroy cancer. Think of a heat seeking missile that is sent out to destroy a jet fighter. The infrared light emission given off by the jet engines would be the antigen. The DC would be the sensor that detects the infrared emission, targets it and then guides the missile to the heat source. The T cells and NK cells would be the

missile's explosive devices that would destroy the enemy jet fighter. This is a simple analogy of the complex and elegant tumour destroying function of the immune system.

To sum up why DCs are at the centre of cancer vaccine development, they are able to present T cells and NK cells to tumour cells, and once they are encoded with a tumour associated antigen, they will retain a memory of the transaction. The DC's memory makes it protective against the recurrence of tumours.

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Preparation

Antigens are found circulating in the blood and on the surface of tumour cells.[23] Antigens are harvested from the patient by drawing blood, or in cases where the tumour is accessible, a tissue sample will be taken. DC precursor cells are contained in the blood or tissue sample and they are cultivated and matured with cytokines such as Interleukin 4 (IL-4),[24] which will activate the T cells, and IL-15 to activate the NK cells.[25] It is interesting to note that NK cells also help the DCs mature.[26] The tumour associated antigen (TAA) is used to pulse the DC and activate it. There are numerous cultivation methods used to isolate the antigens from the blood or tissue samples. One involves repeatedly freezing and thawing the cells. This will mimic necrosis (cell destruction) which will isolate the TAA. Another method is to expose tumour cells to UV light, or gamma irradiation, which will mimic apoptosis (cell death) and isolate the TAA. Another method is to oxidise the tumour cells with hypochlorus acid which will provoke rapid necrosis.[27]

Once the DCs are cultured, encoded with the TAA, and activated along with the T cells and NK cells, the vaccine is ready for injection into the same patient who provided the antigen and immune cells. Generally, the vaccine is given in a series of injections with periods in between shots. The rest period provides time for the DCs, T cells and NK cells to further mature and proliferate. Because the vaccine is using live cells, results improve with time.

Scientific evidence

DCVs are being studied in vitro with cancer cell lines and in vivo in animals and human patients. A clinical trial in Korea was done with breast cancer patients and kidney cancer patients. The vaccine was well tolerated by all patients. NK activity was induced 60% of the patients.[28]

A clinical trial in China administered DCV to colorectal patients. The results were that the DCV extended the disease free period and generated longer survival rates. There was in increased cytotoxic (cancer cell destructive) response observed in 57% of the patients.[29] Another study with 100 colorectal cancer patients demonstrated that more than 70% of the patients experienced improvement in strength, sleep, appetite and weight. Adverse effects, including fever, loss of appetite, joint pain and skin rash were experienced in the 30% of the patients but, these effects were mild and the vaccine was well tolerated.[30] Another study conducted in China showed that DCVs have anti-tumour effects on bladder cancer cells.[31]

A group of researchers in Israel tested a DCV on patients with myeloma. They observed that the disease stopped progressing in 66% of the patients from several months up to two years after vaccination.[32]

Let me bring to your attention three very encouraging clinical trials. They are especially important because they were done with patients presenting pancreatic cancer, lung cancer and metastatic melanoma. The stage IV 5-year survival rate for pancreatic cancer is 2%, lung is 4% and melanoma is 15%.[33] Lets start with the pancreatic cancer study. It was one of the large study using data from seven treatment centres in Japan from 255 patients who had inoperable pancreatic cancer. The multicentre study concluded that DCVs are well tolerated, adverse reactions are mild, and they may improve outcomes in patients who are concurrently receiving chemotherapy or radiotherapy.[34] The study on lung cancer was interesting because lung cancer is not generally considered an immune-sensitive cancer. In the study with just a few non-small cell lung cancer receiving DCV, a potent immune response was observed.[35] The trial with metastatic melanoma was conducted in the United Kingdom. All of the patients tolerated the vaccine well. Most of

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the patients experienced mild adverse effects including skin irritation at injection site and flu like symptoms. All of the patients experienced a good immune response. There was measurable tumour reduction in 12% of the patients and tumour stabilisation in 16% of the patients.[36]

A clinical trial conducted in Lithuania was done including patients with prostate, kidney and bladder cancer. There was an improved immune response in 70% of the patients and a partial or complete remission in 20% of the patients.[37] This study, like the others, confirm that DCVs are important today in the treatment of cancer.

Conclusion

Oasis of Hope has been developing and improving immunotherapies over the last four decades. We have observed that allogeneic immunotherapies, in which cells are donated from a person other than the patient, provide limited results; whereas autologous immunotherapies, where the cells are harvested from the patient, cultured and then infused or injected back into the patient, have produced much better outcomes. We have not observed full remissions that can be attributed to our DCV alone. Instead, we consider our DCV to be an excellent adjuvant therapy because it is effective at inducing an anti-cancer immune response, and in many cases measurable reduction in the size of the tumour. We are encouraged by the number of clinical trials on DCVs being conducted at centers. These studies confirm our observations that DCVs:

- Induce cancer cell death through apoptosis[38]
- Inhibit tumour cell growth[39]
- Can reduce tumour size significantly through necrosis[40]
- Can inhibit metastases[41]

We suggest that further studies need to be done to improve outcomes with DCVs. These studies will lead to breakthroughs in DCV development. We have identified a number of ways that we can improve our DCV. Methods could be developed that would enable DCVs to better activate NK cells and suppress T reg cells.[42] Baylor Research Institute suggests exploring the use of mild chemotherapies, such as clophosphamide (Cytoxan®), to suppress T reg cells before injecting the DCV.[43] Another possibility is harvesting tumour associated antigens from fibroblasts (part of the tumour connective tissue) in addition to the antigens on the cell surface. [44] There is also research that suggest that utilising pharmaceuticals, such as colchicine, during the culturing of the DC may enhance its efficacy.[45] Phytochemicals, such as shikonin, may also enhance tumour immunogenicity of DCVs as well.[46]

Results from new clinical trials are being published every year and we will monitor them carefully. We are committed to continue our research efforts and believe that our DCV will become one of the most effect vaccines.

Glossary of terms

Antigen: Any substance that causes the body to make an immune response against that substance. Antigens include toxins, chemicals, bacteria, viruses, or other substances that come from outside the body. Body tissues and cells, including cancer cells, also have antigens on them that can cause an immune response. These antigens can also be used as markers in laboratory tests to identify those tissues or cells.

Antigen-presenting cell: A type of immune cell that boosts immune responses by showing antigens on its surface to other cells of the immune system. An antigen-presenting cell is a type of phagocyte. Also called APC.

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Antigen-presenting Ccell vaccine: A vaccine made of antigens and antigen-presenting cells (APCs). APCs boost an immune response by presenting antigens on their surfaces to other cells of the immune system. Also called APC vaccine.

Dendritic cell: A special type of immune cell that is found in tissues, such as the skin, and boosts immune responses by showing antigens on its surface to other cells of the immune system. A dendritic cell is a type of phagocyte and a type of antigen-presenting cell (APC).

Dendritic cell vaccine: A vaccine made of antigens and dendritic antigen-presenting cells (APCs).

Lymphocyte: A type of immune cell that is made in the bone marrow and is found in the blood and in lymph tissue. The two main types of lymphocytes are B lymphocytes and T lymphocytes. B lymphocytes make antibodies, and T lymphocytes help kill tumour cells and help control immune responses. A lymphocyte is a type of white blood cell.

NK cell: A type of immune cell that has granules (small particles) with enzymes that can kill tumour cells or cells infected with a virus. An NK cell is a type of white blood cell. Also called natural killer cell and NK-LGL.

Pathogen: Any disease-producing agent, especially a virus, bacterium, or other microorganism.

T cell: A type of white blood cell. T cells are part of the immune system and develop from stem cells in the bone marrow. They help protect the body from infection and may help fight cancer. Also called T lymphocyte and thymocyte.

T reg: A type of immune cell that blocks the actions of some other types of lymphocytes, to keep the immune system from becoming over-active. T regs are being studied in the treatment of cancer. A T reg is a type of white blood cell and a type of lymphocyte. Also called regulatory T cell, suppressor T cell, and T-regulatory cell.

Vaccine: Any preparation used as a preventive inoculation to confer immunity against a specific disease, usually employing an innocuous form of the disease agent, as killed or weakened bacteria or viruses, to stimulate antibody production.

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