Immunotherapy As a Treatment Option for Patients With Pancreatic Cancer

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Abstract

Pancreatic cancer is one of the worst forms of cancer that can develop in an individual. Traditionally, chemotherapy is administered but it has very limited success. Using the immune system to treat the cancer is very enticing and many studies have been conducted to attempt to harness the body's own mechanisms to defeat the cancer. It seems that in order to properly treat the tumor a two pronged approach must be used. First, the immune system must be stimulated to react to the tumor and attack it. A possible cytokine that can be utilized is interferon alpha, which could result in a proliferation of T cells, but also appears to cause severe side effects. This can be overcome by introducing the interferon virally. Second, immune suppression must be overcome. This can be accomplished by using antibodies to destroy regulatory (CD-25) T cells that would ordinarily prevent a T cell response. However, care must be taken to avoid inducing autoimmunity.

Introduction

Pancreatic cancer is one of the worst forms of cancer that can develop in an individual. A growing tumor in the pancreas can grow for fifteen years before it metastasizes, at which point it becomes symptomatic. It is a silent killer growing inside the body. Less than 1 % of patients diagnosed with pancreatic cancer survive even five years, with a median survival of four to six months (Warshaw, Fernandez-del Castillo, 1992). Pancreatic cancer is resistant to traditional chemotherapy treatment and therefore even once the cancer is detected, the treatment currently available to patients is mildly effective at best (Michel, Gress, 2013).

Surgery, as well has proved only mildly effective. Researchers found that less than 10% of patients survived five years after surgical removal of the tumors. This failure has been attributed to small amounts of cells that are left behind that prove to be lethal. In fact, 28% of patients have been found to have circulating tumor cells in the blood and the prevalence increased with the cancer stage (van Heerden, et. al. 1981). This would indicate that surgery alone is not effective and other treatments should be sought.

Recently, a new chemotherapy (FOLFIRINOX) was proposed which showed promising signs, giving patients a longer survival time than that associated with the traditional chemotherapies. However that advantage didn't translate into actually curing the disease, just prolonging the patients' lives by a few months. In addition, this drug showed an increased toxicity, which limited its use to only the few patients that can tolerate it (Conroy, et. al. 2011).

It is therefore obvious that new approaches must be sought towards defeating this cancer. Prolonging the lives of its victims is not an end goal. Rather, a way of ridding them of the disease altogether is required. It is therefore obvious that new approaches must be sought. Recent research has shown promising results in the field of immunology which could indicate some new treatment approaches geared towards eliminating this disease.

Adaptive immunological protection is provided to our bodies via two pathways. B cells provide extracellular or humoral immunity and create antibodies while T cells provide intracellular or cell mediated immunity. Both of these pathways are crucial in order for our bodies to be able to fight off even the "simplest" infections. If these systems are compromised, even a common cold can prove fatal as is evidenced by the HIV virus.

Innate immunity is provided by mechanical barriers such as skin and epithelial linings and cellular protection by macrophages, other phagocytes and natural killer cells. Macrophages phagocytose antigens into a phogosome and upon receiving signals provided by certain cytokines, kill the antigens that are engulfed by combining the phagosome with a lysosome. The lysosome contains toxic chemicals that can degrade the antigens. Natural killer cells respond to infected cells and destroy them before more cells become infected.

Adaptive immunity is provided primarily by B cells and T cells. B cells create a number of antigen receptors. Some of these receptors can be secreted as antibodies and others are attached to the cell surface and function as B cell antigen receptors. The first receptor that the B cell creates is the IgM receptor, which is closely followed by the IgD receptor. These receptors are very specific and can detect sequences of polysaccharides as well as peptide antigens and respond to them. The B cells can then produce different antibodies depending on the nature of the antigen in order to better combat it. The other antibodies that they can produce are IgA, IgE, and IgG. IgA is produced in response to mucosal infections and has the ability to cross mucous membranes. IgE is primarily produced in response to allergens and activates mast cells to release histamine. IgG is one of the primary antibodies used to fight infection due to its utilization of many mechanisms to assist in destruction of pathogens. It can coat antigens (opsonization) in order to assist in their phagocytosis, as well as directly neutralizing them by binding to their surface.

T cells are subdivided into helper T cells and cytotoxic T cells. These cells are distinguished by certain biochemical markers on their surface. The helper T cell has a CD-4 molecule on its surface, while the cytotoxic T cell has a CD-8 molecule on its surface. The CD-4 T cell is further subdivided into Th1 helper T cells and Th2 helper T cells. The Th1 helper T cell is supportive of a CD-8 cytotoxic response and help the cytotoxic T cells lyse infected cells. However the Th2 helper T cell is not supportive of a CD-8 cytotoxic response and promotes immune suppression. All T cells can only recognize antigen by their T cell receptors if the antigen is presented by other cells via a molecule called MHC (Major Histocompatibility Complex) which is present on the other cells. Certain cells are specialized towards presenting antigen to the T cells. This is accomplished by the cell engulfing the foreign material (phagocytosis), degrading it and presenting peptide fragments to the T cell. The cells that are specialized towards presenting antigen are known as antigen presenting cells. These can be dendritic cells, B cells and macrophages. Dendritic cells are especially proficient at antigen presentation therefore, the presence of mature dendritic cells is important in order to present the antigen to the T cells. The CD-4 helper T cells recognize antigen presented via MHC class II which is primarily found only on these specialized antigen presenting cells. On the other hand, the CD-8 cytotoxic T cells recognize antigen presented by MHC class I which is present on almost all cells of the body.

The amazing thing about the immune system is its diversity. Because of the various genes that code for the T cell receptors, the amount of combinations that the T cell can use is astronomical. Therefore, not only can the receptors be incredibly specific, but there can also be a tremendous amount of diversity. The big problem that the T cells and the B cells face is how to "know" how to react to foreign antigen and not react to self-antigen and cause autoimmunity. Autoimmunity is when the immune system targets and starts killing the body's own cells. The way that the body solves this problem is by negative selection. This consists of a rigorous test that T cells undergo in the Thymus and B cells undergo in the bone marrow during development. The cells are exposed to self-antigen. If they recognize self-antigen with high affinity they undergo apoptosis and are not permitted to mature. If however they recognize self-antigen with only low affinity, they are permitted to mature and perform their function.

In addition to regulation at the T cell and B cell development stage, there are certain helper T cells that ensure that T cells do not react to self-antigen. These are known as regulatory T cells and help shut down the T cell response at the end of an adaptive immune response and also ensure that T cells that escaped negative selection in the Thymus can be prevented from attacking healthy tissue and causing auto-immunity. There are also receptors on the T cells themselves that prevent auto-immunity. One of the primary receptors is the CTLA-4 receptor. When it is engaged, even if the T cell is recognizing antigen, the T cell is prevented from mounting a response (Abbas, et. al. 2012).

It has been thought since the 1950s that one of the functions of the immune system is to prevent the occurrence of cancer in the body. This theory is known as immune surveillance. This theory is supported by the high incidence of cancer in immune-compromised hosts such as patients with HIV and AIDS (Goedert, et. al. 1998) as well a high incidence of tumor formation among patients taking immunosuppressive medications (Sanchez, et. al. 2002). This would indicate that there is some role in the prevention of tumors by the immune system. It has therefore been assumed that there must be some antigens that cancerous cells express that are recognized by the immune system. In fact many tumor antigens have been identified. In some cases the antigens are normal proteins that are usually only present during a particular stage of the cell's life but, because of the tumor are now present during other stages as well. They can also be normal proteins that are merely over expressed in the cancer cells. Other antigens that have been identified are mutated proteins that are present in the diseased cell. However, due to the fact that even immune-competent hosts have developed cancer, it would seem that the immune system is not highly adept at fighting these tumors. Can the immune system be induced to fight against pancreatic cancer?

Discussion

Samples of pancreatic tumors from 80 surgically resected tumors were studied for signs of immune cell presence, particularly CD-4 and CD-8 T- cells. Some patients had one and not the other while some had both or none. They noticed that from the patients that had both CD-8 and CD-4 T cell infiltration, the overall survival rate was much higher than those that were missing one or both of these cells. In addition it was apparent that the depth to which the cancerous cells were able to penetrate in the pancreas was much less for those patients with high levels of both CD-4 and CD-8 T cells. It would seem that the presence of the T cells indicates that the T cells are suppressing the cancerous cells and preventing them from infiltrating further. The researchers thus concluded that a high level of T cells in the affected pancreas positively correlates to an increased survival rate (Fukunaga, et. al. 2004).

What can be used to attract the T cells? Pancreatic cancer patients treated with Interferon alpha after undergoing surgery to remove part of the pancreas were studied. Interferon alpha is a cytokine produced by the body and is used in the immune response to infected cells. When a cell is infected it produces various cytokines that cause downstream signaling. This downstream signaling refers to signals that the antigen presenting cells receive by these cytokines and increase the antigen presentation to the T cells. This is an obvious benefit because additional antigen presentation will lead to an increased T cell response. Introducing Interferon alpha in addition to the standard chemotherapy increased the length of

Kaplan-Meier survival statistics

Time	Overall survival (95% CI)	Disease-free survival (95% CI)
One year	95% (91%–98%)	67% (60%–74%)
Two years	64% (56%–72%)	52% (44%–60%)
Three years	64% (56%–72%)	52% (44%–60%)
Five years	55% (46%–65%)	52% (44%–60%)
Follow-up (months)		
Mean ± SD	31.9 ± 24.6	29.7 ± 25.9
Median (range)	21.8 (4–86)	16.0 (3.9–86)

Median survival could not be calculated as 29 of 43 patients (67%) are still alive.

CI = confidence interval.

Table 1:

Survival statistics of patients undergoing interferon alpha therapy (Picozzi, et. al. 2003).

survival for pancreatic cancer patients. 88% percent of patients undergoing the interferon therapy were still alive after two years and 55% survived to the five year mark (Tables 1 and 2). This is a significant increase as the normal 2 year rate of survival for patients being treated with standard chemotherapy alone is 40%. The theory is that as interferon is introduced, tumor antigen presentation is upregulated and therefore more T cells can infiltrate the area and fight the cancer. The study noted however that further research was still needed (Picozzi, et. al. 2003).

However this study was conducted using patients that had already had surgery. Only 20% of pancreatic cancer patients have operable tumors and therefore it is apparent that other methods must be sought out as well.

Some big drawbacks of using interferon-based chemotherapy are the side effects. A recent study of 28 pancreatic cancer patients undergoing this treatment showed an increase in toxicity resulting in a decrease in white blood cells (leukopenia) and a decrease in neutrophils (neutropenia). Although the toxicity was reversible and no patients died, an important consideration is the effect the medication has on overall quality of life. The study found that although the overall quality of life decreased, it wasn't a significantly larger decrease than those patients being treated with the standard chemotherapy. However, it is noteworthy that some of the patients being treated with this therapy needed to be hospitalized with vomiting, abdominal pain and dehydration (Katz, et. al. 2011).

In response to this systemic toxicity, scientists have proposed infecting cancer cells with adenoviruses that express Interferon alpha in order to restrict the effects of the interferon localy rather than systemically. In studies of other cancers, viruses have been shown to exhibit promising effects. The virus can be induced to specifically target cancer cells and not healthy cells by using a tumor specific promoter called cyclooxygenase 2 which is over expressed in cancerous cells. In the case of the adenovirus proposed treatment for pancreatic cancer, after the virally infected cell goes through its replication cycle in the infected host cell, it produces a "death protein" causing cell death so as to release viral particles and infect neighboring cells (Kuruppu, Tanabe, 2005). Although previously pancreatic cancer has shown resistance to adenoviruses, that has been attributed to a lack of coxsackie-adenovirus receptors on the cell surface. The newly developed adenovirus has been coaxed to bind to alternate cell surface receptors namely arginine-glycine-aspartic fiber and Ad3 receptor. When the virally infected cell dies it releases interferon and can thus be contained locally and not cause widespread toxicity and dangerous side effects. This treatment thus combats the cancer in two ways. The virus itself kills cancerous cells, and the interferon

Reported outcomes in patients with resected pancreatic head adenocarcinoma

First Author, year of publication, institution	Adjuvant therapy	No. of patients	Median overall survival (months)	Two-year actuarial survival
GITSG, 1985	EBRT + 5-FU	21	18	43%
GITSG, 1987	EBRT + 5-FU	30	21	43%
Whittington, 1991, Univ Pennsylvania	EBRT + 5FU + mitomycin	20	16	43%
Foo, 1993, Mayo Clinic	EBRT + 5-FU	29	23	48%
Spitz, 1997, M.D. Anderson	EBRT + 5-FU	19	22	44%
Yeo, 1997, Johns Hopkins	EBRT + 5-FU	99	21	44%
EORTC, 1999	EBRT + 5-FU	60	17.1	37%
Sohn, 2000, Johns Hopkins	EBRT + 5-FU (variety)	366	19	39%
Nukui, 2000, Virginia Mason	EBRT + 5-FU, CDDP, IFNα	17	*	84%
Picozzi, 2003, Virginia Mason	EBRT + 5-FU, CDDP, IFNα	43	*	64%

* The median has not been reached for IFNα group survival as 29 of 43 patients (67%) are still alive in the current group.

GITSG = Gastrointestinal Tumor Study Group; EBRT = external beam radiation therapy; 5-FU = 5-fluorouracil; CDDP = cisplatin; IFNα = interferon alpha.

Table 2:

Survival outcomes for pancreatic cancer patients undergoing various treatments (Picozzi, et. al. 2003).

that is released induces immunological resistance to the tumor. (Armstrong, et. al. 2012).

There is another problem that must be dealt with in treating any cancer using immunotherapy; the problem of immunosuppression. As the tumor grows, it changes the local environment from one of immunogenicity (favorable to destruction by the immune system) to one of immunotolerance (unfavorable to destruction by the immune system). As mentioned earlier, CD-8 and CD-4 Th1 T cells are extremely important in proper immune function. However as the disease progresses there is a larger presence of Th2 T cells, ineffective CD-8 T cells and perhaps most significantly, an increase in regulatory T cells which aid in immune suppression by expressing CTLA-4 receptors and secreting immunosuppressive cytokines like TGF-β, IL-4 and IL-5 (Ikemoto, et. al. 2006). There is also an increase in myeloid derived suppressor cells. These cells suppress both the innate and the adaptive immune response (Gabitass, et. al. 2011). In addition, rather than there being an infiltration of M1 - classically activated macrophages - which phagocytose and destroy antigens, there is an infiltration of M2 macrophages which are associated with tissue repair. They therefore remodel the matrix and in fact enhance the tumor and assist in its growth (Schmeider, et. al. 2012) (figure 1).

Therefore, to truly mount an immunological assault on the cancer, these obstacles must be overcome. A way must be found in which to cause an infiltration of Th1 helper T cells, classically activated macrophages and a decrease in the number of regulatory T cells and myeloid derived supressor cells in the area of the tumor. Recent research has been exploring possible options.

Using antibodies that specifically target the CD-25 marker on regulatory T cells, researchers depleted a splenic cell suspension containing T cells, of the regulatory T cells within. They injected

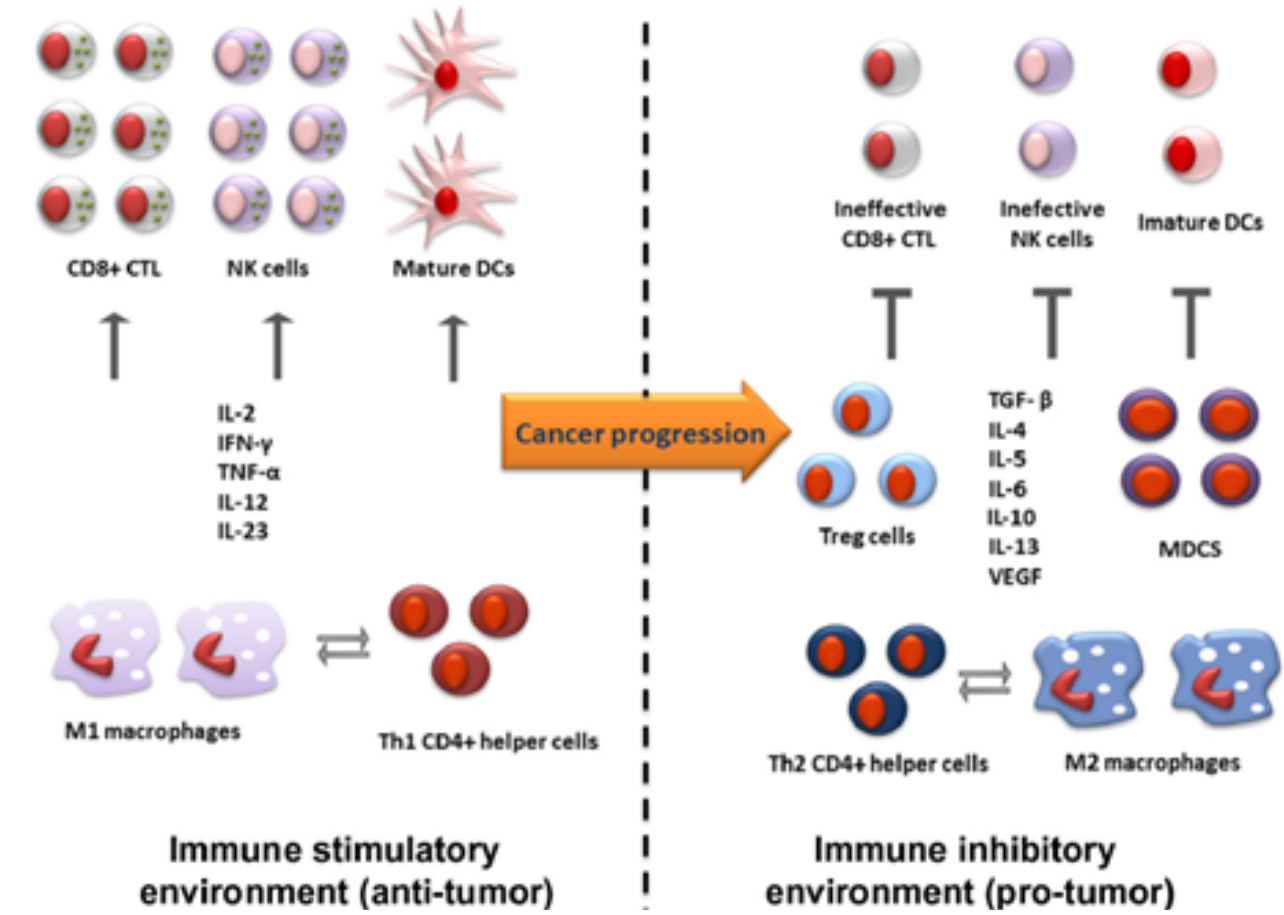

Figure 1.
Changes in the tumor microenvironment during cancer growth (Sideras, et. al. 2014)

nude mice (a strain of mice that due to a genetic mutation have no thymus and therefore have no T cells), with this sample and later with leukemia. They noticed that the tumors first grew and then regressed in most of the mice, allowing the mice to live long term (more than 80 months). Most of the mice that were injected with a non-depleted splenic cell suspension died of the tumor within 40 days. Furthermore, when the mice were reinjected with larger doses of leukemia the immune system mounted an even stronger response and rejected the leukemia much more vigorously. This indicated that the mice had become immune to the cancer. The conclusion of the researchers was that regulatory T cells prevent other T cells from mounting an immune response. They also injected ordinary mice with anti CD-25 antibody and leukemia suspensions. These tumors also grew and then regressed within 1 month in more than 90% of the mice whereas all the control mice, injected with ordinary antibodies (not specific to CD-25), died within 1 month indicating that even normal mice were able to mount a response once the regulatory T cells were depleted (Shimizu, et. al. 1999).

It seems that depletion of the CD-25 T cells allows activated, anti-tumor CD-8 and CD-4 T cells to infiltrate and kill the tumor. In a clinical trial of breast cancer patients treated with Daclizumab, a drug containing anti CD-25 antibodies along with an anti-cancer vaccine a marked decrease in CD-25 T cells was observed (Rech, Vonderheide, 2009). However at the time of this report only three patients were analyzed, and it is therefore premature to declare this treatment a success. In addition, Dr. Vonderheide, one of the researchers in this clinical trial, declared a potential conflict of interest due to his involvement in developing the included cancer vaccine administered along with the Daclizumab. More research on pancreatic cancer as well as other cancer patients still needs to be conducted before this treatment can be assumed to be effective.

However, regulatory T cells have been associated with prevention of autoimmunity. Previously, in studies done where T cells were removed, autoimmunity was generated. It was, however, unclear whether removing T cells caused autoimmunity because of a lack of regulatory T cells or because of a profound lack of any T cells which then allowed microbial infections to occur. Recently, however a study was conducted in which specifically regulatory T cells were removed from mice. Autoimmunity soon developed in various forms such as diabetes, thyroiditis, and autoimmune gastritis (Sakaguchi, et. al. 2001). Any treatment that attempts to eliminate regulatory T cells should therefore try to limit the treatment locally to the site of the tumor or cancerous cells in order to prevent autoimmunity in other parts of the body.

Conclusion

Although pancreatic cancer is one of the most difficult cancers to treat, a multi-pronged approach to treating this disease immunologically would perhaps lengthen the survival time and maybe even cure this disease. A possible approach to treating this disease could include interferon alpha alone, or virally, in combination with anti CD-25 antibodies. This would attract T cells to the site of the cancer while at the same time limiting the amount of regulatory T cells that ordinarily prevent an anti-cancer response. Care must be taken however, to ensure patients do not suffer significant loss of quality of life. In addition a balance must be struck between eliminating regulatory T cells while still avoiding autoimmunity.

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