Current Tactics Employed in Cancer Vaccines and Their Progress

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Cancer is one of the leading causes of death in the US, and the American Cancer Society predicts that there will be approximately 1,660,290 new cancer cases and 580,350 cancer-related deaths in 2013. A wide variety of cancer vaccines including protein and peptide-based, DNA and RNA-based, tumor cell and tumor cell lysate-based, and vector-based vaccines are currently being tested in clinical trials to try and boost the cytotoxic T lymphocyte (CTL) response in humans. Most protein and peptide-based vaccines have shown weak immunogenicity in previous research and in clinical trials. However, one therapeutic protein-based vaccine known as sipuleucel-T and two types of preventive protein-based vaccines have proven successful in clinical trials. Tumor cell-based vaccines have several advantages over the other types of cancer vaccines. This is mainly due to their ability to target many different antigens (usually proteins) simultaneously, including antigens besides human leukocyte antigens (HLAs). Vaccines employing tumor cells can use either allogeneic or autologous tumor cells with allogeneic being more beneficial because of the simplicity involved in engineering them. Vector-based vaccines mostly utilize poxviral vectors containing recombinant genes to induce an inflammatory response in a host against the proteins within the vector. A problem with vector-based vaccines is that neutralizing antibodies can be developed against the vector by a host. Genetic cancer vaccines consisting of strictly DNA or RNA have proven partially effective in generating an immune response in hosts, but still have a long way to go in clinical trials before they can be used in humans. Future research should look at helping the immune system to identify tumor antigens along with differentiating between somatic and tumor cells.

INTRODUCTION

A staggering 23% of all deaths among all age groups in the United States are caused by cancer, making it second only to heart disease in the number of deaths that it causes each year in the country. However, cancer happens to be the leading cause of death in men and women between 40 and 79 years of age (Seigal, Naishadham, & Jemal, 2013). Even though death rates have decreased by 20% since their peak in 1991, cancer still remains a major health problem. Recent assessments have indicated that the incidence of cancer is expected to be over one million in 2013. Mortality rates due to cancer are also expected to be high in 2013, with over half a million deaths predicted. Lung and bronchus, breast, prostate, and colorectum cancer are expected to cause approximately 50% of these new cancer cases among men and women (Seigal, Naishadham, & Jemal, 2013). With the exception of melanoma, thyroid and liver cancers, the incidence rates for the majority of cancers have steadily diminished in men and women since 2001-2002 (Seigal, Naishadham, & Jemal, 2013). This trend is attributable to the efficacy of cancer therapies, better diagnostic equipment, and a greater budget being available for further cancer-related research. While cancer treatments may be more effective at the present time than they were in the past, the continually high incidence and mortality rates suggest that these treatments still need to be improved not only for the United States, but also globally. Thus,

either current tactics against cancer need to be modified to become more efficient in controlling aspects like tumor growth, or, new approaches need to be considered to further reduce cancer incidence and mortality. As an example, one potential new strategy to treating cancer could be the use of cancer vaccines in conjunction with prescribed cancer drugs.

A variety of cancer therapy methods have already been designed in response to its prevalence and high death rate. One current method of interest in treating cancer involves immunotherapy, which is used to try to create a specific immune response that either prevents or treats cancer. There are two types of tumor immunotherapy: passive and active. Passive tumor immunotherapy pertains to tumor-specific cells or antibodies being transferred to a patient. In contrast, active tumor immunotherapy works to induce an immune response through the process of immunizing a patient with tumor cells or tumor antigens. The latter refers to preventive and therapeutic vaccines (Vergati et al., 2010). Well-constructed vaccines have the ability to affect only tumor cells while other therapies like radiotherapy and chemotherapy can be used to destroy tumor cells at the cost of losing some normal cells (Berzofsky et al., 2004). Compared to other types of cancer treatments, vaccines seem to be the only treatment with the capacity to prevent cancer or greatly reduce the amount of cancerous tumors (Vergati et al., 2010).

Cancerous cells share many similar self-antigens with normal, non-cancerous cells of the body because they are derived from their host. This makes it difficult to merely destroy tumor cells and not innocuous cells (Berzofsky et al., 2004). The human immune response to these self-antigens, or tumor associated antigens (TAAs), is weak because the immune system thinks of

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these TAAs as antigens from normal cells (Vergati et al., 2010). Therefore, antigens must be found that are strictly associated with cancer cells and not host cells. A large majority of possible tumor antigens are located away from the surface of tumor cells, making them unreachable to antibodies (Berzofsky et al. 2004). A multitude of therapeutic vaccines have been suggested for treating cancer in the past twenty years in order to boost the weak immune response induced by TAAs. Some of these vaccines employ nucleic acids, use synthetic peptides and proteins, incorporate microbial vectors, or utilize actual cancer cells (Vergati et al., 2010). A similar factor among all of these vaccine strategies is the attempt to trigger a cytotoxic T lymphocyte (CTL) immune response in order to lyse tumor cells (Berzofsky et al., 2004; Vergati et al., 2010). The vaccines allow CTLs to dramatically increase the amount of identifiable tumor antigens by the immune system. Cytotoxic T lymphocytes also have a key role in rejection of organ and tissue transplants, which is comparable to the role tumors play as foreign cells in a host (Berzofsky et al., 2004). Each type of cancer vaccine possesses its own unique advantages and disadvantages (Rosenberg et al., 1998). The purpose of this review is to describe the structure of these vaccines and determine their efficacies in both treating and preventing cancer by looking at clinical trials and other studies.

Vaccines Using Proteins and Peptides

Protein and peptide vaccines are a couple of vaccines that have been long sought after due to the wide range of possibilities they offer at combating cancer. Moreover, new discoveries of the peptides and proteins that make up tumor antigens have sparked new interest in this area of vaccines. Since T-cells recognize peptide fragments introduced by major histocompatibility complex (MHC) molecules, a considerable amount of time has been put into developing vaccines using antigenic peptides as the vaccinating antigens (Disis et al., 1996). Protein and peptide based vaccines utilize components such as heat-shock proteins (HSPs), peptides and agonist peptides, among other agents (Vergati et al., 2010). Heat shock proteins are transcribed by the heat shock transcription factor 1 (HSF1), and research has shown that they are important in evoking innate and adaptive immunity. A general feature of all major HSPs is the capability to guide peptides and to assist T-cell responses by activating dendritic cells (DCs). Heat shock proteins are released from the cells and attach to antigen-presenting cells (APCs) primarily through the CD91, CD40, and LOX-1 receptors. Following uptake by APCs, the peptides are then translocated to MHC class-I molecules, which move to the cell surface allowing these peptides to be visible to CD8+ T cells (Fan et al., 2012). Heat shock proteins operate as endogenous, non-microbial, danger molecules that can regulate the expression of co-stimulatory and antigen-presenting molecules on DCs. They also stimulate the secretion of pro-inflammatory cytokines, leading to the activation of natural killer (NK) cells and other immune cells (Fan et al., 2012; Prohaszka et al., 2012).

Another area of interest in peptide vaccine therapy is agonist epitopes. By making peptides with agonist epitopes and pulsing (packing) dendritic cells with these peptides, the immunogenicity of a self-antigen can be enhanced. Evidence has shown that the PSA-3A agonist ("A" for agonist) shows increased binding to the MHC class I A2 allele, in addition to increased stability of the peptide-MHC complex when compared to native prostate specific antigen (PSA)-3 (Terasawa et al., 2002; Yokokawa et al., 2007). Also, human T-cell lines manufactured with this (PSA)-3 agonist peptide have shown the capacity to lyse carcinoma cells (containing the original PSA) of the human prostate. Therefore, agonist peptides could turn out to be quite useful in cancer vaccines employing peptides if they continue to produce strong levels of T-cell activation while also destroying cancer cells in an MHC-restricted manner (Terasawa et al., 2002)

Equally important, protein and peptide-based vaccines have two main advantages over the use of tumor cells. One benefit of these vaccines is that the production, storage, and distribution process is much more effective than with tumor cells. Additionally, administering tumor-specific antigens is preferred to tumor cell preparations as well. The preference is due to the fact that many self-proteins provide no curative benefit and have the ability to induce an autoimmune response. However, there are a few drawbacks to this approach. First of all, single proteins and single epitopes do not provoke significant immune reactions in patients. Besides that, tumors can avoid the immune system by means of antigen mutation or loss. Lastly, these vaccines are limited to a small number of patients. Moreover, the protein and peptide-based vaccines have a poor potential of prompting a balanced activation of CD4+ and CD8+ cells, which is thought to be vitally important for effective, long-lasting antitumor immunity. The majority of epitope-based vaccines are able to produce responses that efficiently kill tumor cells, but do not have a long lifespan when CD4+ T-helper cells are depleted. Protein-based vaccines have the ability to create more efficient CD4+ responses (MHC class II-restricted), but at the drawback of less effective induction of CTLs (Table 1). Using a combination of multiple epitopes in the same vaccine or longer peptides could help to solve nearly all of these issues. Also, because of the weak immune response generated by peptides, a possible way to resolve this issue would be the use of peptides in combination with adjuvants. Another option for counteracting the inadequate response of peptides would be to load peptides onto dendritic cells, a technique that was previously mentioned (Vergati et al., 2010).

Peptide and protein vaccines have already progressed to clinical trials, and one drug known as Sipuleucel-T has been FDA-approved. Composed of APCs cultured with a fusion protein, Sipuleucel-T is made to stimulate T-cell immunity to prostatic acid phosphatase (PAP). PAP is an antigen found in most prostate cancers, but not in non-prostate tissue (Small et al., 2006). In the case of metastatic castrate-resistant prostate cancer among men, Sipuleucel-T has shown evidence of the ability to reduce the risk of death (Kantoff et al., 2010; Kono et al., 2012; Small et al., 2006). Another peptide vaccine that is currently in trials is DPX-

Vaccine Type	Advantages	Disadvantages
Protein/Peptide-Based Vaccines	1. Production, storage, and distribution are more effective than in other types of cancer vaccines. 2. Administering tumor-specific antigens is preferred to tumor cell preparations.	1. Single proteins and single epitopes have a weak immunogenicity. 2. Has a poor potential of prompting a balanced activation of CD4+ and CD8+ cells.
Vector-Based Vaccines	1. Can be made without a great amount of difficulty as compared to other cancer vaccines. 2. Relatively inexpensive to make.	1. Neutralizing bodies can be developed against the vector by the host. 2. Immune responses may be targeted against the viral vector antigens and not against weaker antigens.
Tumor Cell/Tumor Cell-Lysate Vaccines	1. Have the ability to affect only tumor cells, while other treatments like radiotherapy and chemotherapy affect normal cells. 2. Target different antigens at the same time and are not restricted to HLAs.	1. Use of autologous tumor cell vaccines is cumbersome and tumor samples are often unavailable. 2. Sometimes not useable for large-scale vaccine production.
DNA/RNA Vaccines	1. Well permitted by patients and are safe, making clinical trials easier to perform. 2. Use of RNA-based vaccines showed that T-cell responses did not react with normal tissues.	1. Has not shown much promise in generating a strong immune response. 2. Aren't as effective in humans as they are in small animals.

Table 1. Strategies employed in cancer vaccines.

0907. DPX-0907 comprises a polynucleotide-based adjuvant and a universal T helper peptide. With seven HLA-A2 (specific allele group on HLA-A locus) restricted peptides from tumor-associated antigens, this particular vaccine is capable of involvement in numerous cancer pathways such as tissue invasion and metastasis. Easily made and relatively safe, DPX-0907 also has the advantages of long-term stability, undemanding storage, and uncomplicated changeability (Berinstein et al., 2012).

Despite the success of sipueleucel-T, preventive medicine remains the most effective method. To illustrate this fact, preventive vaccines developed for the hepatitis B virus and the human papilloma virus are examples of successful protein-based vaccines. Studies have shown that vaccinating against the hepatitis B virus (HBV) and the human papilloma virus (HPV) is beneficial in stopping the spread of these viruses, which in turn helps with the prevention of cancer caused by these viruses (Chang et al., 1997, 2009; Marty, Roze, Bresse, Largeron, & Smith-Palmer, 2012; Westra et al., 2013).

Vaccines Using Tumor Cells or Tumor Cell Lysates
Another type of cancer vaccine involves the use of tumor cells. There are several advantages offered by tumor cell vaccines that are lacking in other types of cancer vaccines. For instance, while producing an immune response, tumor cell vaccines can direct attention towards an array of different antigens at one time while not being restricted to HLAs (Table 1). With the wide assortment of MHC class I and class II epitopes being processed or in other words recognized by the immune system, it is also likely that both the innate and adaptive immune responses will be activated (Vergati et al., 2010). Once placed in the body, tumor cell vaccines

stimulate the immune system by exposing it to certain antigens on the tumor cells. The body then recognizes these antigens as foreign and targets any other cells in the body that present the same foreign antigens.

There are two distinct types of tumor cell vaccines: autologous (patient-specific) and allogeneic (non-patient specific) (Groot et al., 2005). Autologous tumor vaccines are essentially tumor cells that have been removed from a patient, irradiated so they cannot produce more tumor cells, and then placed back into the same patient to help combat the cancer. Allogeneic tumor vaccines, on the other hand, are taken from one patient, irradiated, and then placed into another patient to produce an immune response. There are multiple advantages to using allogeneic cells instead of autologous cells. To begin with, the supply to tumor-associated antigens (TAA) is almost limitless since non-patient specific cells originate from well-defined cell lines. The use of cell lines makes large-scale production of allogeneic vaccines a tangible reality as well. Essentially, the benefit of allogeneic tumor cells over autologous tumor cells is that the need for tailor-made vaccines for individual patients is eliminated. This cuts down on labor and the delivery process, which cuts down on the overall cost of the treatment (de Gruijl, van den Eertwegh, Pinedo, & Scheper, 2008; Vergati et al., 2010).

Both autologous and allogeneic tumor vaccines have advanced to at least clinical trials in humans. OncoVax is an autologous tumor vaccine used to treat colon cancer. Studies have shown that treatment with OncoVax leads to a 20% reduction in cancer progression. GVAX is another drug that has been studied as a potential treatment for prostate cancer. GVAX is a granulocyte–macrophage colony-stimulating factor (GM-CSF) gene-transfected tu-

mor cell vaccine, meaning that the tumor cells in GVAX have been modified to secrete GM-CSF (Nemunaitis et al. 2006). Research has shown that treatment by GVAX produces an immune response signified by infiltration of neutrophils, CD4+ T cells, and apoptotic cells in the area of injection. Additionally, large amounts of GM-CSF are secreted at the injection site in patients receiving the GVAX vaccine. In theory, the secretion of GM-CSF increases the antigen presentation of tumor-specific antigens (TSAs) and TAAs. This facilitates the immune system's ability to thwart tumor cell growth on account of its increased efficacy to identify and attack the antigens of the tumor cells (de Gruijl et al., 2008; Salgia et al., 2003; Vergati et al., 2010). While treatment with tumor-based vaccines is promising, there are still many obstacles that stand in the way of tumor vaccines becoming a viable option for cancer patients.

Vaccines Using Vectors

An alternative vehicle for vaccine delivery is a vector, which exposes antigen-presenting cells to certain recombinant genes via delivery by bacteria, yeasts, or viruses. When these vectors with recombinant genes are injected into a host, they can produce an inflammatory response against any proteins associated with the vector. Furthermore, this may result in a specific immune response being initiated against the recombinant genes brought into the host by the vector (Vergati et al., 2010). When looking at transferring certain proteins into the host, studies have shown that delivery of a recombinant protein by a vector induces a stronger immune response than if proteins were administered with adjuvants (Larocca & Schlom, 2012; Vergati et al., 2010). The type of vector used can also play a major role in the type of immune response produced towards TAAs as bacterial, yeast, and viral vectors each possess their own unique set of features. The ideal vector-based vaccine should be able to promote a sufficient amount of both innate and adaptive responses so that the preferred cells can be activated in the immune reaction (Vergati et al., 2010). Compared to the majority of other immunotherapy methods, viruses with recombinant genes can be made without a great amount of difficulty and are relatively inexpensive to make (Larocca & Schlom, 2012). A possible disadvantage with vectors is that the host could possibly begin to make antibodies against them that render the vectors ineffective (Table 1) (Berzofsky et al., 2004; Larocca & Schlom, 2012).

The most commonly used vectors in vaccines are poxviral vectors. The different types of poxviruses include viruses based on the original vaccinia virus used in eradicating smallpox, other orthopox viruses, and members of the avipoxvirus family. Poxviruses are made up of double-stranded DNA, with replication of the viruses occurring in the cytoplasm of infected cells. Since this replication occurs in the cytoplasm, the viruses are harmless to the host because no viral based genetic sequences can be placed in the genome of the host cell (Larocca & Schlom, 2012; Vergati et al., 2010). Nevertheless, a major factor of most viral vectors involves modifying them to be non-replicating (Vergati et al., 2010). If the viral genomes were not modified to be non-duplicating, the virus could possibly infect and destroy human cells, especially in immunocompromised patients. Poxviruses are also able to hold a number of genes, making them good candidates for recombinant genes. Once transgenes are in the host, expression of a transgene within cells can lead to processing of the tumor antigen by both major histocompatibility complex pathways, eventually sparking the initiation of T helper cells (Larocca & Schlom, 2012).

A weakened avipoxvirus known as Fowlpox is commonly used as a booster vaccination in conjunction with another more effective vaccination because of the weak immune response it generates on its own (Vergati et al., 2010). In a phase II clinical trial, a majority of patients with prostate cancer who were undergoing radiation therapy were given a priming vaccine (recombinant vaccinia) and a monthly recombinant fowlpox booster vaccination, which produced a prostate specific antigen (PSA) immune response to the vaccines (Gulley et al., 2005). Multiple other studies further support that recombinant fowlpox vaccines given as a booster vaccination after a priming vaccination with recombinant vaccinia are more effective than using each vector alone.

The recombinant genes placed in vectors tend to be expressed at high levels in the host, which in turn helps most vector-based vaccines to generate strong immune responses. An additional beneficial feature of poxviruses is they can be altered to express certain T-cell co-stimulatory molecules and cytokines, as well as a recombinant gene. These co-stimulatory molecules and cytokines have been found to ultimately help with tumor recognition by CTLs because certain co-stimulatory molecule signals are needed for activating T cells (Vergati et al., 2010).

In a pilot study that examined the therapeutic potential of co-stimulatory molecules, 25 patients with metastatic carcinoma were treated with the PANVAC-V as a prime vaccination (a recombinant vaccinia virus) and PANVAC-F as a booster vaccination (a recombinant fowlpox virus). The PANVAC-V vaccine also contains three co-stimulatory molecule transgenes along with the CEA (carcinoembryonic antigen) and MUC-I (Mucin 1) genes. The PANVAC-V treatment resulted in high patient tolerance and immune reactions to MUC-1, CEA, or both in 9 of 16 patients who were tested following vaccination. It was concluded that this particular vaccine combination is rather safe and can be linked to CD8+ and CD4+ specific immune responses (Gulley et al., 2008).

Another viral vector called the adenovirus has also been studied in clinical trials. One specific gene that has been used in association with adenoviral vectors is the p53 gene, a tumor suppressor gene that assists in preventing cancer through regulation of the cell cycle. In one study performed in order to test the immune and clinical effects of a new adenoviral vector vaccine (containing wild-type p53 gene) in patients with extensive-stage small cell lung cancer, 57.1% of patients showed a p53-specific T cell response following repeated vaccinations every two weeks (three vaccinations for most patients). A clinical response was observed in one patient following vaccination, but when subsequent chemotherapy was performed a total of 61.9% of patients showed a clinical response. In this study, a clinical response was defined as

having at least a 30% decrease in the sum of the longest diameter of target lesions (partial response) or the disappearance of all targeted lesions (complete response). Based on these results, it was determined that the adenovirus vaccination alone might not be the answer to treating cancer, but instead a combination of a vaccination along with other cancer therapies (chemotherapy) might be more effective (Antonia et al., 2006). Combination treatments involving a poxviral vaccine followed by nilutamide hormone treatment have also showed therapeutic promise in some studies (Madan et al., 2008). Additionally, vaccines using vectors can be constructed to target certain genes as previously mentioned. One such gene is the HER2/neu gene, which is overexpressed in 30 to 40% of breast cancers. Utilizing a prime-boost vaccine strategy involving a DNA vaccination along with an adenoviral vaccination (both used the gene for rat HER2/neu), it was discovered that this vaccine protocol (injected into mammary fat pad or intravenously) could prevent growth of a breast cancer cell line (HER2/neu expressing) (Wang et al., 2005). However, this kind of tumor cell prevention remains to be seen in humans when using this same cancer vaccine treatment combination. An immense problem surrounding the use of adenoviruses regards the limited amount of clinical responses they are known to produce (Fan et al., 2012). Even so, some studies suggest that adenoviral vectors may provide more of an upside than protein pulsing methods (Chiriva-Internati et al., 2002). As for bacterial and yeast vectors, current evidence suggests they are not as effective in studies as viral vectors in recent years (Vergati et al., 2010).

Vaccines Using DNA/RNA

Another means of transport for vaccines involves the use of plasmid DNA, which is used to transfect a live organism and produce antigens within that organism. The plasmid, a tiny and circular piece of bacterial DNA, is genetically constructed to encode a specific antigen or antigens of the pathogen under the influence of a mammalian promoter. When the plasmid DNA is injected into the host (subcutaneously, intradermally, or through the muscle), it can penetrate the nucleus of the cells that it has infected (APCs, monocytes, etc.). The host cells can then read the plasmid DNA and utilize it to produce the antigens of the pathogen. The process by which the plasmid DNA affects the immune system is not entirely known, but it is thought that the bacterial DNA activates the innate immune system via interaction with the Toll-like Receptor 9 (TLR9) found on APCs.

A distinct characteristic of DNA vaccines is their potential to introduce proteins to the immune system of the host, allowing them to trigger CD4+ T cells along with CD8+ cytotoxic T cells. Evidence suggests that DNA-based vaccines have shown to be well tolerated by patients. Additionally, they are safe, making clinical trials much easier to perform (Fioretti, Iurescia, Fazio, & Rinaldi, 2010). When first tested in the early 1990's, the vaccines showed no detrimental side effects towards subjects and appeared safe, but still did not show much promise in generating a strong immune response (Table 1). This was especially true in humans

at the time, but since then many adjustments have been made to try and increase the immunogenicity and overall effectiveness of these vaccines including the way in which the plasmid DNA is delivered into hosts. For example, DNA vaccine delivery using electroporation causes a rise in the production of antigens because of the increased amount of DNA plasmids taken up by the cells (Ferraro et al., 2011). During a phase 1 clinical trial, electroporation was used in conjunction with a bivalent DNA vaccine containing plasmids expressing either carcinoembryonic or human epidermal growth factor receptor 2 antigens. Following vaccination, no patients displayed a cell-mediated immune response against the CEA and HER2 cancer antigens (Diaz-Montero et al., 2013). Additionally, DNA plasmids have also been used in prime-boost protocols with certain viral-vector vaccines and have shown promise in some murine studies producing both cellular and humoral immunity against tumor cells (Wang et al., 2005). However, like the DNA-adenoviral vaccine combination treatment discussed previously, this type of immune response has been almost non-existent in humans.

Cytokine gene adjuvants (immunological agents added to vaccines) also have been utilized in an attempt to improve the immune response generated by DNA based vaccines. In one study involving a DNA-based prostate-specific antigen (PSA) vaccine used in a prostate cancer model (rhesus macaques and mice), cytokine gene adjuvants were used to enhance the PSA host immune response. This vaccine combined with interleukin (IL)-2 cDNA (complementary DNA) was able to dramatically improve the PSA-specific antibody responses in both the murine and primate models. Nevertheless, the strength of immune response produced by cytokine gene adjuvants was higher in the mice than it was in the macaque model. The study hypothesized that this difference in response was due to the fact that human cytokine complementary DNA was used to vaccinate the primate models in this study, possibly affecting the potency of the cytokine genes in primates. Also, it is possible that DNA-based vaccines (even when modified) are nowhere near as effective in bigger animal models (primates and humans) than they are in smaller animal models (Kim, Yang, & Dang, 2001). In another trial, 22 patients exhibiting stage D0 (one of four levels in the late stage) prostate cancer were treated with a DNA vaccine encoding the human Prostatic acid phosphatase (PAP) antigen along with a granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant. In this, nine patients developed PAP-specific CD4+, CD8+, or both CD4+ and CD8+ T-cell immune responses following vaccination. These results seem to suggest that a DNA vaccine used in conjunction with an adjuvant may provide more effective immune responses in humans than DNA vaccines alone (McNeel et al., 2009).

In addition to DNA-based vaccines, there are also vaccines made from mRNA that are injected (mRNA introduced into non-cancerous cells) into a host by means of transfection. The source of mRNA for these vaccines can come from prostate specific antigen peptides, a person's own tumor RNA, or even another person's tumor RNA. A benefit to this type of vaccine is that the mRNA

is transiently transfected (only expresses its protein briefly) into a host, meaning it cannot integrate into the genome of that host. The mRNA used in these vaccines is typically plentiful in the tumor cells that the vaccines are designed to destroy. The mRNA coding for the tumor-associated antigen is specifically introduced into dendritic cells where it is then made into proteins. Once the immune system has made these proteins and the specific antigens are expressed, a cytotoxic T-cell response is then produced against these antigens (Vergati et al., 2010).

Furthermore, human dendritic cells infected with renal tumor RNA produced strong CTL responses against both primary and metastatic renal tumor antigens. In one particular study, research was conducted to see if DCs injected with autologous renal cell carcinoma (RCC) RNA would produce a multitude of immune responses against previously unidentified tumor antigens (Heiser et al., 2001). In a phase 1 clinical trial also involving renal tumor RNA, 10 patients with confirmed metastatic RCC Stage IV were immunized with renal tumor RNA-transfected DCs. Six of the 7 evaluable patients in this study displayed an increase in tumor-specific T cells, which reacted against multiple renal TAAs. However, an important finding in this study was the fact that the T-cell responses did not react with any normal renal tissues (Su et al., 2003). These findings allude to the possibility that vaccines utilizing RNA, if improved to provide the necessary immune response, could potentially become the safest and most efficient vaccines available against cancer. These results suggest that vaccines utilizing RNA could arguably become the most efficient and safest vaccines available against cancer in subsequent years. This is provided that they are properly improved to deliver the necessary immune response for reducing and destroying tumor cells. A combination therapy involving RNA-based vaccines could also prove effective.

CONCLUSIONS

Even with the numerous vaccine strategies developed specifically for preventing and treating cancer, this disease remains a major problem throughout the world. Whether peptide/protein-based, tumor-based, vector-based, or DNA/RNA based vaccines, the majority of cancer vaccines tested have been unable to enhance the body's immune system to a point of where these vaccination strategies could be effectively utilized in a hospital setting for treating cancer patients. In fact, the only vaccine for treating cancer currently approved by the FDA is Sipuleucel-T (Provenge), which is used for men who have metastatic prostate cancer. Preventive cancer vaccines have been developed for the hepatitis B virus and human papillomavirus (type 16 and 18), and the FDA has approved these vaccines as well. Many clinical trials involving cancer vaccines are ongoing in the hope that more therapeutic vaccines can be found. However, there are still only three types of therapeutic or prophylactic cancer vaccines that can be used outside of clinical trials. After more than 20 years of researching and studying cancer vaccines, there is no doubt that much more research needs to be done. Future research should focus not only on

boosting the immune system but also finding a way to manipulate the immune system to attack tumor cells through the detection of tumor antigens. Furthermore, an effective cancer vaccine should provide long-term memory to the immune system to help achieve partial, if not complete, remission of the cancer. One particular factor impeding antigen detection is the fact that tumor cells disguise themselves as normal cells. To fix this obstacle, further research needs to be conducted to discover ways in which tumor cell antigens can be made more recognizable by the immune system. Another problem with creating vaccines for cancer is that tumor cells have many of the same antigens that normal cells do, making it extremely hard to direct the immune system to detect and attack only tumor cells while sparing somatic cells. Therefore, scientists and other cancer researchers need to more closely examine the antigens specific to tumor cells and identify some of the key cancer cell antigens so that these antigens can be used to strictly kill cancer cells. Finding ways to eliminate some, if not all, of these challenges could lead to great success in the field of cancer immunotherapy while at the same time minimizing the unfortunate number of cancer cases and cancer-related deaths that continue to plague society today.

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