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Tumor vaccines

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Abstract

Melanoma vaccines are an exciting and increasingly attractive immunotherapeutic approach for malignant melanoma. Vaccines can be used for patients with high risk primary melanoma and regional disease, stages in the progression of melanoma for which there is presently no treatment. They are unique in their potential to prevent cancer in high risk individuals.

Multiple approaches are being followed to develop effective vaccines. It is too early to judge whether any of them effectively slow the progression of melanoma. However, it is clear that vaccines are safe to use, and that they can stimulate immune responses to melanoma in some patients. The specificity of these responses needs to be clarified, and multiple challenges remain to be overcome before effective vaccines to melanoma become available. We must first identify the antigens on melanoma that stimulate immune responses, define the immune effector mechanisms that are stimulated by vaccine immunization and identify those responsible for increasing resistance to tumor growth, devise appropriate ways of constructing vaccines that will induce such responses, and find adjuvants and/or immunodulators that will potentiate desirable immune responses.

Introduction

Active specific immunotherapy with vaccines constructed of tumor antigens is a conceptually attractive approach to treat and possibly prevent cancer. The rationale is that such vaccines may be able to stimulate the immune system to react more vigorously, specificially to cancer cells and thus augment resistance to a patient's tumor. The most convincing evidence that this approach is valid is the fact that tumor vaccines prevent some cancers in animals [1–3]. Preliminary studies suggest that they may slow the progression of some cancers in humans [4–12].

Tumor vaccines have several potential advantages over other immunotherapies for cancer. They can selectively augment immunity to tumor, and thus should have a more potent effect than immunotherapeutic approaches that non-specifically augment immune reactivity, such as BCG, IL-2, and other immunomodulatory and biological response modifying agents. The immunity induced by vaccines has the potential to be long-lasting, as opposed to passive immunotherapy with monoclonal antibodies or adoptive therapy with immune cells, whose effect is transient. Vaccines can stimulate cellular responses, which are thought to be the principal effector mechanism in immune resistance to cancer. Vaccines are relatively non-toxic, so they can be used early in cancer progression, after the surgical removal of the primary tumor, at a stage when chemotherapy and other forms of immunotherapy are not used because of their toxicity. Finally, vaccines are unique in their potential for preventing cancer.

As a result, there is now an increasing interest in the use of vaccines to treat cancer. Much of it is focused on melanoma because it appears to be an

excellent model for the study of cancer vaccines [13]. This review analyzes the current status of cancer vaccines, using our efforts to develop a vaccine for human malignant melanoma as an example.

Rationale for cancer vaccines

It is important to realize that the progression of cancer depends not only on the instrinsic malignant potential of the tumor, but also on the patient's immune response to it. As a consequence, it may be possible to increase resistance to cancer by stimulating specific anti-tumor immunity with a vaccine.

In the case of malignant melanoma, the evidence that immune mechanisms may increase resistance to this cancer consists of the following observations:

1. The growth of melanoma in humans is influenced by host factors. The most dramatic demonstration of this is the spontaneous and complete regression of disseminated melanoma, a rare but well-documented phenomenon that occurs in up to 0.5% of patients [14]. Less dramatic but much more common is the partial regression of melanoma, an event which can be seen in up to 25% of primary melanomas [15]. There can be a prolonged latent period between the removal of a primary tumor and the appearance of metastatic lesions; during this time tumor cells are present in the body, but are prevented from growing by some mechanism [14]. Melanoma cells may circulate in the blood without metastatic spread, and conversely, metastatic disease may be present without a known primary [14], suggesting that the cancer cells, metastatic and primary respectively, have been destroyed. All of these events indicate that host factors can slow tumor growth and even cause regression. Unfortunately, these factors are not sufficiently potent to be able to prevent ultimate disease progression in most cases.

Two experiments of nature clearly demonstrate that the body has mechanisms available to achieve the goal of melanoma immunotherapy, i.e., the selective destruction of pigment cells. These are vitiligo and the 'halo' nevi phenomenon, conditions in which pigment cells are selectively destroyed without damage to nearby unrelated cells. The cause of pigment cell destruction in these conditions is unknown, but it is suspected that an anti-pigment cell immune response is the common denominator [16]. The presence of hypopigmentation favorably influences the prognosis of melanoma [17]; this suggests that the host mechanisms that selectively destroy normal pigment cells can also slow the progression of melanoma.

2. Tumors including melanoma cells can express antigens that differ qualitatively and/or quantitatively from those on normal adult tissues [18]. These antigens are sufficiently different from those on normal adult cells to be recognized as foreign and to trigger humoral and/or cellular immune responses in persons with cancer. At least in some cases, these responses have the capacity to destroy cancer cells *in vitro* [19].

3. Stimulation of anti-tumor immunity can increase resistance to tumor, while immunosuppression can enhance tumor growth. As indicated below, active immunization to melanoma vaccines can prevent melanoma in mice [1, 20]. Conversely, immunosuppression of animals by thymectomy or with anti-lymphocyte serum leads to more aggressive melanoma growth. The same is true in humans. The incidence of melanoma in immunosuppressed patients is four to eight times greater than one would expect [21]. These observations indicate that immune mechanisms have a real impact on tumor growth, rather than being interesting but clinically irrelevant epiphenomena, and that they can be manipulated to give clinically desirable results.

Vaccines prevent cancer in animals

The most convincing evidence that vaccines can increase resistance to cancer is that they prevent some tumors in animals [2, 3, 22, 23]. For example, we have shown that mice actively immunized to vaccines composed of whole B16 melanoma cells or to soluble, partially purified antigens extracted from the tumor will be protected against a challenge of syngeneic B16 melanoma cells that rapidly

kills all non-immunized mice [1]. This observation has been confirmed by others [20, 24]. The protective effect is specific [1], indicating that it is mediated by an immune mechanism.

Tumor protective immunity is associated with the development of specific antibodies and a cellular (delayed type hypersensitivity) immune response to B16 melanoma [1, 25]. The antibodies are directed to several surface antigens that are selectively expressed by B16 melanoma cells [25].

It is difficult to induce the rejection of already established tumors with vaccines [24]. However, this can be accomplished if tumor mass is reduced by surgery or chemotherapy, or if immunotherapy is initiated soon after tumor inoculation.

The implication of these findings is that tumor antigen vaccines may be able to increase resistance to cancer in humans, and that the approach will be most effective in patients with minimal disease. This, in turn, suggests that vaccines should be used early in the evolution of tumors or after tumor load has been reduced by surgery or chemotherapy. The clear ability of vaccines to prevent cancer in animals suggests that ultimately the most effective applications of vaccines may prove to be the prevention of this cancer in high-risk individuals.

Key issues in design of cancer vaccines

The ideal cancer vaccine should be safe, effective against a broad range of tumors of the same histological type, sufficiently potent to require only a few immunizations, simple to manufacture in a reproducible manner, and stable for a prolonged period of time. No such vaccine is available. Some of the key issues that must be considered in the construction of cancer vaccines are discussed below.

1. Selection of tumor antigen

In order to be effective, vaccines should be constructed from tumor antigens that are: a) able to induce clinically effective immune responses in humans; b) expressed on the tumor to be treated; and c) located at a site on the tumor where they can be seen by, and can interact with, immune effector mechanisms; i.e., on the external surface of tumor cells. Antigens that satisfy these requirements have not yet been identified.

2. Tumor antigen immunogenicity

The most basic requirement for a cancer vaccine is that it contains tumor antigens that can stimulate a strong and clinically effective antitumor immune response in humans. Little is known about the identity of such antigens. Most tumor antigens have been defined with monoclonal antibodies that are raised by immunizing animals with human tumor cells. These identify antigens that are recognized as foreign in animals, but they provide no information about the immunogenic potential of the antigen in humans. It is possible to identify tumor antigens that are immunogenic in humans by using human monoclonal antibodies, allogenic or autologous antisera, or human T cell clones. By applying these techniques, several antigens on malignant melanoma that are immunogenic in humans and cross reactive among melanomas have recently been identified. These include the gangliosides GD2, O-acetylated GD-3, and GM-2, a 250 kD glycoprotein, a urinary tumor associated antigen, and a 48 kD antigen. We have used immunoprecipitation SDS-PAGE analysis to compare the pattern of melanoma antigens defined by pre- and post-vaccine treatment sera in the same patients to directly identify antigens that can induce immune responses in humans. These have turned out to be cell surface antigens with molecular weights of 200+, 150, 110, 75, and 38 kDs [26]. The 200+ and 110 kD antigens are of particular interest for vaccine construction because they appear to be the most immunogenic, and seem to be selectively expressed on melanoma [27]. The functional effect of the immune responses induced by these antigens is still not known. Further application of these approaches will hopefully lead to the definition of panels of immunogenic tumor antigens that are broadly expressed on different melanomas and can be used to construct purified second generation vaccines.

3. Tumor antigen heterogeneity and modulation

For vaccine immunotherapy to be effective, the immune responses induced must be directed to antigens expressed by the tumor being treated. Unfortunately, the pattern of tumor antigens expressed by cancers of the same histological type in different individuals is variable. There is also variation in the pattern of tumor antigens expressed by different tumor nodules in the same individual, and by different tumor cells in the same nodule [28]. In addition, as a result of antigenic modulation, the profile of tumor antigens expressed by a tumor during its progression may be altered by the immune response of the host [29]. As a consequence, it is unlikely that vaccines prepared from a single tumor antigen will be effective against a broad range of tumors of the same histological type. For the same reason preparing autolegors vaccines from a patients own tumor cells does not ensure it will be effective against other tumor cells in the same patient. However, many tumor antigens are commonly expressed on many, although not all, tumors of the same histological type. The expression of different common antigens is complementary, so tumor cells that lack a particular antigen may express other types of tumor antigens [28]. Thus, a viable strategy for circumventing the antigenic heterogeneity of tumors is to construct polyvalent vaccines containing a broad range of widely expressed immunogenic tumor antigens.

4. Potentiating vaccine immunogenicity

Tumor associated antigens are weak immunogens. As an example, antibody and/or cellular response to melanoma in patients with this cancer are the exception rather than the rule [29–31]. Methods must be developed to boost the activity of those antigens that are capable of inducing immune responses in humans.

Three general strategies are available to augment the immunogenicity of antigens: 1) Modifying their physical or biochemical properties or presenting them in multimeric units that are more immunogenic. 2) Administering the antigen together with an adjuvant. 3) Using immunomodulators to increase the ability of the host to respond to the antigen and to drive the responses that are induced in a desired direction. These approaches are not mutually exclusive. It is likely that combinations will have an additive or synergistic effect.

Examples of antigen modifications that have been attempted to augment the immunogenicity of melanoma antigens includes physical aggregation of the antigens, neuraminidase treatment [8], and infecting the melanoma cells with live nonpathogenic viruses [5, 7, 11]. Melanoma antigens have been coated unto liposomes or incorporated into vaccinia virus to enable their presentation in multimeric units. No comperative studies are available to judge the relative efficacy of these procedures.

The classic approach to augmenting the immunogenicity of vaccines is to use an adjuvant. Unfortunately, adjuvants that are safe for humans, such as alum, are not very potent, while potent adjuvants such as BCG or Freund's complete adjuvant can cause severe skin reactions. A number of newer adjuvants have been developed which, it is claimed, retain the potency of mycobacterial adjuvants without toxicity. Full clinical evaluation of these adjuvants in humans is still pending, so their effectiveness is not known.

There is increasing interest in using immunomodulators such as cyclophosphamide, interleukin-2, and other lymphokines alone or in combination in order to more vigorously drive forward immune responses that are stimulated by vaccines or to inactivate suppressor machanisms that may depress the response. The most extensively studied immunomodular is cyclophosphamide (see below). The effect of these agents on the immunogenicity of vaccines in humans is still unclear. However, one concern is that many immunomodulators are toxic in their own right, and/or require repeated administration in a hospital or physician's office, which negates a major attractiveness of tumor vaccines – their safety and ease of use.

5. Stimulation of inappropriate immune responses

Immune responses to tumors are complex both in

their variety and in their effect. While some responses may damage tumor cells and hinder tumor growth, others may protect these cells and enhance their growth [32]. Thus a critical aspect of vaccine development is the identification of beneficial immune responses, as evidenced by a correlation between the presence of such a response and a favorable clinical outcome. As described below, we have found such a correlation between the stimulation of a delayed type hypersensitivity response to vaccine immunization and a delay in tumor recurrence.

An aspect of this problem that has been the object of particular attention is suppressor cell function, which may depress immune responses to active immunization to tumor antigens. Several strategies are available to minimize suppressor cell activity. Immunization with low doses of antigens [33] and intradermal administration of antigens [33] both reduce suppressor cell function. Cytotoxic drugs such as cyclophosphamide administered 2 to 4 days prior to antigen challenge may selectively inactivate suppressor cells and, in animals, can enhance the immunogenicity of vaccines. Studies in patients with advanced melanoma suggest the same may be true in humans [9]. However, in our study of this agent we did not find that it potentiated the immunogenicity of a melanoma vaccine in patients with early (regional disease) melanoma [34]. As a result, we suspect that the vaccine potentiating activity of cyclophosphamide may be restricted to patients with advanced disease, in whom there may be greater disturbance in suppressor cell function.

6. Tumor load

Animal studies indicate that tumor vaccines are most effective if the tumor load is small. The implications of this finding are twofold: a) Vaccines should be used to treat patients with minimal disease. b) The usual criterion used to evaluate the effectiveness of chemotherapeutic agents, i.e., the ability to cause regression of established disease, should not be used as the critical test of tumor vaccine effectiveness, as it may miss important beneficial effects in preventing tumor recurrence. The regression of established melanoma in vaccine-treated patients with disseminated disease has been reported [36, 10, 9], so vaccine may also be active in patients with advanced disease.

Vaccine design strategy

Our strategy for constructing a vaccine for melanoma was designed to circumvent some of the problems described above. Its major elemets are: a) Preparation from a pool of melanoma cells, selected because they express different patterns of melanoma antigens, in order to create a polyvalent vaccine that can circumvent tumor antigen heterogeneity. b) The use of cultured cells to ensure a continued and reproducible supply of material for vaccine preparation and to permit treatment of patients in whom no tumor tissue is available. c) Adapting the melanoma cells to grow in serum-free medium to exclude these highly immunogenic and undesirable proteins from the vaccine. d) Preparing the vaccine from shed material. e) Further purifying the vaccine to deplete HLA antigens. The elements of this strategy and their advantages in relation to other approaches to vaccine design are discussed below.

1. Selection of cells for vaccine preparation

It is clear from: a) the antigenic heterogeneity of tumors, and b) the lack of information about the nature of tumor antigens that stimulate protective immune responses, that cancer vaccines should contain multiple tumor associated antigens. They will then be more likely to contain immunogenic antigens expressed by the tumor to be treated. For this reason we prepared our vaccine from a pool of four different melanoma cell lines, selected because they expressed different patterns of melanoma associated antigens. As described later, this approach was successful in creating a vaccine that contained multiple melanoma associated antigens (MAAs). Most of the MAAs tested for were present in the vaccine, so it is probable that it contains additional MAAs that were not tested for. At least one of the MAAs present in the vaccine was ex-

pressed by each of 23 metastatic melanomas that we immunophenotyped, so the antigen mix in the vaccine seems appropriate for circumventing the antigenic heterogeneity among melanoma.

An alternate strategy for dealing with tumor antigen heterogeneity is to prepare vaccine from autologous tumor cells obtained from the patient to be treated. There are several problems with this approach. The first is that autologous vaccines do not circumvent the problem of tumor antigen heterogeneity. The antigen profile of different tumor cells in the same person is variable. As a consequence, vaccine prepared from tumor nodule A will not necessarily contain the antigens expressed by tumor nodule B in the same individual. Autologous vaccines are impractical, since an individual vaccine must be prepared for each patient. Finally, perhaps the greatest drawback of autologous vaccines is that they require a fair amount of tumor tissue for their preparation, so they cannot be used in the patients with minimal disease who are the best candidates for vaccine immunotherapy, in patients whom no fresh tumor tissue is available, or prophylactically to prevent disease.

Another consideration in selecting tumor cells for vaccine preparation is whether they should be freshly obtained from surgically excised tumor tissue or maintained in tissue culture. Fresh tumor tissue is more likely to express antigens relevant for immunotheraphy. However, its use is impractical for the reason discussed with autologous antigens – the need to make individual vaccine for each patient and the inability to treat patients with no available tumor tissue. Cultured cells, on the other hand, provide an economical and reproducible source of material for vaccine production and enable the preparation of generic vaccines that can be used to treat all patients with the same histological type of tumor, as well as patients who have no tumor tissue available.

The cells used to prepare our vaccine have been adapted to grow in serum-free medium in order to avoid contaminating the vaccine with this undesirable material. FCS proteins are highly immunogenic, and appear to be responsible for many of the immune responses induced by human tumor vaccines prepared from cultured cells [37–39]. This approach seems to have been successful, since no FCS protein can be detected in our vaccine [36]. It should be noted that because FCS protein adheres tightly to cells, simply washing tumor cells or incubating them briefly in serum-free medium will not get rid of the FCS protein.

3. Collection of tumor antigens for vaccine production

A key step in our method of vaccine preparation is to obtain the antigens used to construct the vaccine from surface material shed by the melanoma cells. It is based on the principle that tumor cells rapidly release into the culture medium a broad representation of cell surface components, including tumor antigen [40, 41]. Shed material has several advantages as a source of tumor antigen for vaccine construction. It is easily collected, in a manner that can be scaled up for commercial production. The tumor antigens are partially purified, as they are separated from the bulk of unrelated cellular components that are in the cytoplasm and are poorly released in the short collection periods used. As a result the shed material is enriched in surface antigens [41], which are most relevant for immunotherapy. The antigens can be repeatedly harvested from the same cells, reducing culture requirements.

There are other methods for obtaining tumor antigens for vaccine preparation. Most involve the use of whole tumor cells. The cells are either used intact [12, 9], or after they have been broken up mechanically with detergents or other treatments, or lysed with non-pathogenic viruses such as vaccinia [5, 11, 7], Newcastle disease [5], or VSV [38]. The bulk of the material in such vaccines is derived from the cytoplasm, rather than from the surface of the cells, and is thus irrelevant to vaccine activity. The presence of irrelevant antigens in the vaccine may diminish immunogenic activity through antigenic competition. Vaccines prepared from whole cells are contaminated with nuclear material that creates increasing safety concerns as the vaccine is used to treat less advanced disease. Vaccines prepared from viral oncolysates present the additional

safety concern that they contain live viral particles, although this is counterbalanced by the possibility that the viral components increase the immunogenicity of the tumor antigens.

Ideally, vaccines should be constructed from pure tumor antigens. Unfortunately, this approach seems premature, since the tumor antigens that should be used for this purpose – antigens that can induce strong tumor protective immune responses in humans – are not known.

4. Vaccine purification

As it is prognosed from shed antigens, the vaccine is already fairly purified as it is separated from the bulk of cytoplasmic and nuclear cellular material which is poorly shed. The vaccine is put through a further simple purification procedure intended to deplete HLA antigens, which involves detergent treatment and ultracentrifugation. Much of the material shed by melanoma cells is in fragments or vesicles. These can be broken up with detergent to release tumor antigens in a soluble form that remains in the supernatant after ultracentrifugation [41]. However, as others have found and we confirmed [41], ultracentrifugation still sediments detergent treated transplantation antigens, providing a simple way of separating HLA from tumor antigens.

Properties of vaccine

The vaccine contains multiple melanoma-associated antigens (MAA), including the 240+ kD proteoglycan antigen (described by S. Ferrone and Dr. R. Reisfeld), the p97 kD MAA (described by Dr. K. Hellstrom), the 26, 29, 95, 116 kD antigen (described by Dr. H. Koprowsky), and 75, 95, 120, 140, 150, and 240 kD MAAs defined by polyclonal antisera raised in our laboratory. A number of the antigens in the vaccine have been shown to be immunogenic in humans (see below). It is free of fetal calf serum proteins [36]. The vaccine can be made reproducibly, as three batches prepared on different occasions contained the same pattern of melanoma associated antigens [36]. For use, the vaccine is bound to alum as an adjuvant.

Steps in evaluating a tumor vaccine

The clinical evaluation of cancer vaccines involves three major steps: a) Evaluating safety in humans. b) Evaluating biological activity or immunogenicity – can the vaccine stimulate antibody/cellular immune responses to the tumor, and if so, how often. c) Evaluating clinical effectiveness – can the vaccine slow the progression of the tumor.

Safety

Our vaccine has been administered to over 200 patients. There has been no toxicity other than transient urticaria at the injection site [43]. A small asymptomatic granuloma, caused by the alum, remains at the injection site for several months. There has been no skin ulceration.

Other cancer vaccines have been administered to several thousand patients. No toxicity due to the intrinsic properties of the vaccines themselves has been reported. The enhancement of tumor growth resulting from the stimulation of inappropriate immune responses, or the induction of autosensitization, are theoretical risks. No clear evidence of these phenomena has been reported.

The side effects of vaccine immunotherapy have been caused, not by the vaccines themselves, but by the adjuvants (such as BCG, Freund's complete, or incomplete adjuvant) given to enhance the activity of the vaccine. These can cause persistent ulceration at the site of injection, and fever. It can be expected that side effects will also result from some of the immunodulators now being considered to boost vaccine immunogenicity. As the use of tumor vaccines is extended to patients with milder disease or to prevention of disease in healthy but high-risk individuals, the concern for safety will increase. Specific areas of concern include the presence of nuclear material or live viral particles in some vaccine formulations.

In summary, tumor vaccines appear to be rela-

tively safe, and are associated with fewer side effects than conventional chemotherapy or other forms of immunotherapy.

Immunogenic properties of vaccine

This is an important attribute of a vaccine's activity, as it is unlikely that a vaccine will be clinically effective if it cannot induce or augment immune responses to the tumor being treated.

In addition, the type and frequency of immune response induced by a vaccine may provide early clues as to its clinical effectiveness. For this reason, an important area of investigation is the correlation between parameters of the immune response to vaccine immunization and clinical outcome.

Evaluation of a vaccine's immunogenic potential involves measuring the antibody and/or cellular response (preferably both) it is able to induce in humans, and determining whether these responses are directed to tumor as opposed to unrelated antigens. In the case of our vaccine, we found that antibody (measured by protein A-sepharose immuno recipitation) and cellular (measured by DTH response to skin tests to vaccine) immune responses to melanoma were induced or augmented by immunization in 24% and 51%, respectively, of the first 55 patients treated [43].

It is important to realize, when studying the immunogenic potential of vaccines, that the incidence of immune responses detected will depend heavily on the sensitivity of the assay used. As an example, we detected vaccine-induced antibody response to melanoma in 61% of 26 sequential patients using a recently improved immunoprecipitation SDS-PAGE analysis assay [26, 27] but in only 11% of these patients using our former assay.

The antibodies induced by our vaccine are directed to one or more cell surface antigens with approximate molecular weights of 200+, 150, 110, 75, or 38 kDs [26]. The most immunogenic antigens are the 200+ kD and the 110 kD molecules [27]. Both are melanoma-associated, as they are preferentially expressed on melanoma cells. Both antigens were expressed on four of five human melanomas, but on only two of 12 control cell lines. These antigens are not related to HLA molecules based on their molecular weight, nor to FCS proteins, as antigen binding is not blocked by an excess of cold FCS.

The DTH response to the vaccine was carefully analyzed, as it correlates with an improved clinical outcome. DTH responses were induced in a variable (18% to 54%) proportion of patients, depending on the immunization procedure used. The most immunogenic regimen involved using alum as an adjuvant [35].

The DTH response appears to be selectively directed to melanoma. None of the first 17 patients with a DTH response to the vaccine reacted to skin tests to an equal amount of a control vaccine prepared from allogeneic lymphocytes pooled from five different normal donors [43]. Since pooled lymphocytes are a rich source of a broad spectrum of class I and II MHC antigens, it is unlikely the DTH response induced by the melanoma vaccine is directed to MHC antigens. Only 1 of the 24 patients with a strong DTH response to the vaccine reacted to skin tests with concentrated complete culture medium used to grow melanoma cells, making it unlikely that the DHT response is induced by a culture medium contaminant.

The vaccine also stimulates a T cell response which can be measured *in vitro* and which is selectively directed, at least in part, to melanoma. *In vitro* cytotoxicity was measured sequentially before and after vaccine immunization in 18 pts by Dr. Aliza Adler using a direct 4 hr ^{51}Cr release assay. Periphereal blood lymphocytes (PBL) were the effector cells, and a panel of the four melanoma cell lines used for vaccine preparation (M14, M20, HM54, SK-Mel-28) and four control cell lines (melanoma [SK-Mel-23], colon carcinoma [SK-CO1], K562, Daudi) were the targets. Enhanced cytolytic activity to melanoma following vaccine immunization was detected with a frequency that depended on the melanoma cells used as targets. The most sensitive target was M20 melanoma. Vaccine treatment enhanced cytolytic activity to these cells in all patients studied. In two patients, this response was directed selectively to melanoma, i.e., cytolytic activity was strongly augmented to M20 and weakly to M14 and SK-Mel-28 melanoma cells, but was

unchanged against control cells. In the other patients PBL killed both melanoma and some non-melanoma cells, suggesting that immunization stimulates both melanoma selective and non-selective cytolytic cellular responses.

The vaccine can stimulate a cellular immune response to a patient's own tumor. Dense (> 15 cells/high power field) infiltrates of tumor infiltrating lymphocytes (TIL) were more frequent in subcutaneous metastases removed from vaccine-immunized patients (10 of 11 patients, 91%) than in similar metastases removed from non-immunized patients (9 of 22, 41%, p = 0.02) [42].

Clinical effectiveness of melanoma vaccine immunization

Randominzed, concurrently controlled, clinical trials have not yet been carried out with any melanoma vaccine. Consequently, the clinical effectiveness of melanoma vaccines in slowing the progression of melanoma is still uncertain, but there are a number of promising preliminary observations. In advanced disease, we [36] and others [9, 10] have observed occasional regression of established disease. However, as the course of melanoma is erratic it is unclear to what extent these favorable responses are the result of vaccine treatment.

As an alternate approach to examining the impact of vaccine treatment on the progression of melanoma, we have correlated the ability of the vaccine to induce an immune response to clinical outcome. The analysis was conducted in 99 sequential patients with post-surgical stage II (regional disease) melanoma. The DTH response to the vaccine was measured prior to and following the fourth vaccine immunization. There was a relationship between the magnitude of vaccine-induced increase in DTH response and prolongation in disease-free (DF) survival. Median DF survival was 4 years longer in the patients with a strong increase in DTH response to the vaccine than in non-responders (> 65 vs 12 months, respectively). By Cox proportional hazard analysis the relationship between the strength of the DTH response and the increase in DF survival was significant (P = 0.01), and could not be accounted for by differences in desease severity (thickness or level of primary lesion, age, sex, number of involved regional nodes, nodes clinically positive at presentation) or immune status (as evaluated by responses to recall antigens or by sensitization to DNCB). There is also a correlation between the ability of an immunization procedure to stimulate DTH response and delay in tumor recurrence [35].

These results indicate that the DTH response to vaccine immunization correlates with the clinical outcome. This finding suggests that vaccine immunization may be effective in delaying tumor progression in patients in whom the vacccine can stimulate a DTH response.

Prevention of cancer with vaccines

An exciting potential application of cancer vaccines is the prevention of cancer. This is a reasonable possibility, as it can be done in animals (as described earlier). Hopefully, this can also be accomplished in humans. The prevention of cancer may in fact prove easier to accomplish than the treatment of established disease. In mice, it is easier to prevent melanoma with vaccines than to slow its progression once it is present [24]. Similarly, vaccines for infectious diseases are more effective in preventing than in treating disease. If the melanoma vaccines currently being developed prove to be safe to use and effective in patients with established melanoma, it will be reasonable to examine whether they can prevent melanoma in patients at high risk of developing this disease. As a result of the considerable progress that has been made in identifying risk factors for melanoma, such individuals can be identified with a fair degree of precision; this includes persons with large congenital nevi or with familial dysplastic nevi syndrome and a family history of melanoma.

Conclusions

Cancer vaccines are a conceptually attractive meth-

od for treating and possibly preventing cancer, for the following reasons:

1. They can prevent cancer in animals.
2. They are relatively safe to use, and have fewer side effects than conventional chemotherapy and other forms of immunotherapy.
3. They are biologically active, and can stimulate immune responses to cancer in some patients, including responses directed to the patient's own tumor.
4. Preliminary studies suggest they may be able to slow the progression of some cancers in humans.
5. Because cancer vaccines are relatively safe, they are particularly attractive as adjuvant therapy in early disease, a stage in the evolution of most cancers for which there are no effective therapeutic options after surgery.
6. Cancer vaccines are unique in their potential application to preventing cancer.

Key unanswered questions

Researchers should be considering the following topics:

1. The identity of tumor antigens which are immunogenic in humans and which should be used for vaccine construction.
2. The appropriate mix of immunogenic tumor antigens required to construct polyvalent vaccines that can circumvent the antigenic heterogeneity of tumors.
3. The development of appropriate methods for boosting the immunogenicity of vaccines.
4. The identification of vaccine-stimulated host effector mechanisms that are important in protective immunity.
5. The demonstration that vaccines are clinically effective by conducting randomized, concurrently controlled clinical trials.

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