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POINT AND COUNTERPOINT

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Counterpoint. Cancer vaccines: single-epitope anti-idiotype vaccine versus multiple-epitope antigen vaccine

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Abstract Anti-idiotype (Id) vaccine therapy has been tested and shown to be effective, in several animal models, for triggering the immune system to induce specific and protective immunity against bacterial, viral and parasitic infections. The administration of anti-Id antibodies as surrogate tumor-associated antigens (TAA) also represents another potential application of the concept of the Id network. Limited experience in human trials using anti-Id to stimulate immunity against tumors has shown promising results. In this "counterpoint" article, we discuss our own findings showing the potential of anti-Id antibody vaccines to be novel therapeutic approaches to various human cancers and also discuss where anti-Id vaccines may perform better than traditional multiple-epitope antigen vaccines.

Key words Anti-idiotype antibodies · Vaccine · Immune response

Introduction

For many years, immunotherapy has been an appealing option for the treatment of certain types of cancer on the basis of its potential for achieving maximal therapeutic benefit with minimal toxicity. Tumor-specific immunological interventions can be categorized into passive immunotherapy, such as when antitumor antibodies are used, and active immunization to boost or induce a host antitumor response. A third approach is the stimulation of patients' effector cells with cytokines, which is both "active" and "passive". Active immunotherapy can be further subdivided into those approaches that depend on tumor-derived materials such as tumor-associated antigens (TAA) or tumor cells, and methods that do not depend on materials derived or extracted from tumors. Anti-idiotype (Id) manipulation is the major tumor-specific active approach that does not use tumor-derived material to induce antitumor immunity.

Immunotherapy is very effective in certain animal model systems, and it has been used to treat human cancers for several decades [48]. Active immunotherapy of cancer patients with tumor-derived material has been studied by numerous investigators, with positive clinical responses reported. The major problems using tumor material for immunization is that TAA are typically weakly immunogenic. A common explanation for the absence of antitumor immunity is that the immune system has become tolerant to by the tumor antigens. If this is true, steps could be taken to break the existing tolerance. An effective method of breaking tolerance is to present the critical epitope to the now tolerant host in a different molecular environment [50]. While this can be done with well-defined antigens such as haptens, it is impossible with most tumor antigens because they are chemically ill-defined and difficult to purify. Carbohydrate antigens are even more difficult, as they cannot be produced by recombinant techniques.

The immune network hypothesis offers a unique approach to transforming epitope structures into Id determinants expressed on the surface of antibodies. Jan Lindemann in 1973 [26] and Niels Jerne in 1974 [22] proposed theories that describe the immune system as a network of interacting antibodies and lymphocytes. According to this original network hypothesis, the Id anti-Id interactions regulate the immune response of a host to a given antigen. Both Id and anti-Id have been used to manipulate cellular and humoral immunity.

The network hypothesis predicts that, within the immune network, the universe of external Ag is mimicked by idiotypes expressed by antibodies and T cell

This article is counterpoint to the preceding paper by Maruyama et al.

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receptors. According to the network concept, immunization with a given Ag will generate the production of antibodies against this Ag, termed Ab1. This Ab1 can generate a series of anti-Id antibodies against Ab1, termed Ab2. Some of these Ab2 molecules can effectively mimic the three-dimensional structures of external Ag. These particular anti-Id, called Ab2β, which fit into the paratopes of Ab1, can induce specific immune responses similar to the responses induced by nominal Ag. Anti-Id antibodies of the β type express the internal image of the Ag recognized by the Ab1 antibody and can be used as surrogate Ag. Immunization with Ab2β can lead to the generation of anti-anti-Id antibodies (Ab3) that recognize the corresponding original Ag identified by the Ab1. Because of this Ab1-like reactivity, the Ab3 is also called Ab1′ to indicate that it might differ in its other idiotopes from Ab1. This cyclic nature of complementary binding sites and idiotopes is the basis for the approach to idiotope vaccines. Several such Ab2β have been used in animal models to trigger the immune system to induce specific and protective immunity against bacterial, viral (including HIV), and parasitic infections. (Reviewed in [2]). The administration of Ab2β as surrogate tumor-associated Ag represents another potential application of the Id vaccine concept.

Active immunization with tumor-specific Id vaccines has been shown to inhibit the growth of tumor in animal models [23, 46, 47]. A series of studies [35–38] on the effect of anti-Id therapy in a mouse leukemia model L1210 in DBA/2 mice has been described, which has provided us with basic information on B- and T-cell-induced responses, using the anti-Id approach. These investigators generated a number of anti-Id hybridomas against mAb to the L1210 tumor. These anti-Id mAb have been shown to induce tumor-specific DTH, inhibition of tumor growth, CTL, antibodies and T helper cells in this system. These findings are very promising since they demonstrate a cross-reaction of nominal Ag and internal-image Ag for a tumor-associated Ag system at the T and B cell level. In a recent study, the 100% cure of established tumors was achieved in DBA/2 mice by combining anti-Id vaccines with cyclophosphamide, whereas a 50% cure rate was obtained with anti-Id therapy alone [11]. Similar findings have also been obtained when cyclophosphamide (100 mg/kg), administered in combination with Id vaccines to mice bearing 10-day-old, subcutaneous B cell lymphoma (38C13) 1–2 cm in diameter, resulted in dramatically improved survival [7]. An anti-Id antibody was used to induce immunity to simian-virus-40-transformed cells [24]. Mice vaccinated with this anti-Id demonstrated prolonged survival after tumor transfer. The role of Id interactions in regulating the immune response of mice to chemically induced, syngeneic sarcomas has been recently studied [33]. Treatment with anti-Id mAb of mice with the established sarcomas (MCA0-490 and MCA-1511) had significant antitumor activity. Similarly in another recent study, immunization induced immunity to mutant p53 and tumor rejection in mice [41].

Anti-Id responses have been implicated in the induction of antitumor immunity to colorectal cancer [25]. Clinical trials in human colorectal patients [21] with a polyclonal anti-Id raised against the mAb 17-1A, which recognizes a colon-cancer-associated Ag, have shown antitumor antibody responses. In another study it was demonstrated that intradermal injection of 2 mg anti-Id mAb MK2-23, which mimics a high-molecular-mass human melanoma antigen, elicited antitumor antibody responses in melanoma patients [30]. Repeated injections of murine anti-Id mAb were not associated with side-effects. Reduction in the size of metastatic lesions were observed in 7 of the 37 immunized patients. Another 25 patients with stage IV melanoma were immunized with the mouse anti-Id mAb MK2-23, which bears the internal image of the determinant defined by anti-(human high-molecular-mass melanoma antigen) (high-M_r MAA) mAb 763.74. Fourteen patients developed antibodies that were shown by serological and immunochemical assays to recognize the same determinant (or a spatially close one) as that recognized by the anti-(high-M_r MAA) mAb 763.74 and to express the idiotope defined by mAb MK2-23 in their antigen-combining sites. Side-effects that were likely to be caused by bacillus calmetle-Guérin present in the immunogen consisted of erythema, induration, and ulceration at injection sites [31]. Patients occasionally complained of flu-like symptoms, arthralgias, and myalgias. Three patients who developed anti-(high-M_r MAA) antibodies achieved partial responses, consisting of decreases in the size of metastatic lesions that lasted 52 weeks in 1 patient and 93 weeks in 2 others. Survival of the 14 patients who developed anti-(high-M_r MAA) antibodies was significantly longer than that of the 9 patients who did not develop detectable anti-(high-M_r MAA) immunity.

A human monoclonal Ab2 (105AD7) that interacts with the binding site of 791T/36, a mouse monoclonal antibody against the gp72 antigen, was administered to 6 patients with advanced colorectal cancer in a phase I clinical study [40]. Cryopreserved peripheral blood mononuclear cells were tested for in vitro proliferative responses by [^{3}H]thymidine incorporation; plasma samples were tested by an enzyme-linked immunosorbent assay for anti-anti-Id and antitumor antibodies and for interleukin-2. Proliferative responses to gp72-positive tumor cells were seen in 4 of 5 patients tested; parallel in vitro responses to 105AD7 anti-Id antibody were seen in most of these patients. Interleukin-2 was detected in the plasma of 4 of 6 patients after 105AD7 immunization, with peak levels up to 7 units/ml. There was no toxicity related to anti-Id immunization and there were no antitumor or anti-anti-Id antibodies reported.

Advantages of anti-idiotype antibodies over conventional antigen vaccines

The network hypothesis offers still another elegant concept for developing vaccines that is not based on

the conventional approach of using nominal antigen. These so-called anti-Id vaccines or internal Ag vaccines take advantage of the fact that the repertoire of external or nominal antigens is mimicked by Id structures on immunoglobulins and possibly on receptors and products of T cells as well. Thus, with this approach, Id-based vaccines do not contain nominal Ag nor its fragments. This excludes the possibility that Id vaccines would have the same undesired side-effects that are sometimes associated with conventional antigen vaccines.

Besides the increased safety of Id vaccines, these new kinds of Ag have other practical, economical and biological advantages over conventional vaccines. Id vaccines do not depend on the availability of large amounts of pure Ag, which often is a limiting economical factor in vaccine production. By virtue of their being proteins, Id vaccines can be easily manipulated; they can be coupled to potent immunogenic carriers to become T-cell-dependent antigens. Eventually it might be possible to produce fully synthetic Id vaccines using essential sequence information obtained from Id hybridoma Ag.

T-dependent protein vaccines can become a decisive factor in situations where the responding immune system is immature or suppressed. From experimental studies on animals, we know that the response to T-cell-dependent Ag matures earlier than the T-independent response to carbohydrate Ag, and that often a genetically or acquired abnormal immune system responds better to T-dependent Ag than to T-independent Ag.

Finally, data exist showing that an acquired state of tolerance to one Ag form can be broken by using a different molecular form of the same antigenic moiety. This could become an important consideration in a broader context such as in the immunotherapy of cancer patients, who may be immunodeficient or tolerant to their own tumor. In this report, we will discuss various examples where anti-Id antibodies have been used by us successfully in cancer therapy.

Preclinical and clinical trials with anti-Id vaccines

We have generated monoclonal Id cascades for four different human tumor-associated antigens. The first cascade originated from a T cell leukemia/lymphoma-associated antigen [3, 4], the second one from carcinoembryonic antigen (CEA) [5], the third from human milk fat globule (HMFG) membrane antigen [6] and the fourth one from the disialoganglioside GD2 [44]. In each of these cascades, we have produced TAA-mimicking monoclonal anti-Id [3–6, 14, 44]. These monoclonal anti-Id were characterized thoroughly and were capable of generating Ab3 (Ab1′) responses in mice, rabbits and monkeys [8, 9, 43] that recognized the original nominal Ag.

Anti-idiotype vaccine for human colorectal carcinoma

CEA is a tumor-associated antigen expressed on most gastrointestinal adenocarcinomas and is a putative target for cancer immunotherapy. We developed a murine monoclonal anti-idiotype (anti-Id) antibody, designated 3H1, which mimics a specific epitope of CEA. The efficacy of 3H1 as a tumor vaccine was evaluated in a murine tumor model [34]. In this model, the murine colorectal cancer cell line MC-38 was transduced with the human CEA gene and injected into syngeneic C57BL/6 (H-2^b) mice. Immunization of naive mice with 3H1 conjugated with keyhole limpet hemocyanin and mixed with Freund's adjuvant induced humoral and cellular anti-3H1 as well as anti-CEA immunity. Mice immunized with 3H1 were protected against a challenge with lethal doses of MC-38-CEA, whereas no protection was observed when 3H1-vaccinated mice were challenged with CEA-negative MC-38 cells or when mice were vaccinated with an unrelated anti-Id antibody and challenged with MC-38-CEA cells ($P < 0.003$). These data demonstrated that the 3H1 vaccine can induce protective CEA-specific antitumor immunity in this murine tumor model [34].

To demonstrate the efficacy of 3H1 vaccine against established tumors, we performed some preliminary experiments [34]. Mice were injected with 5×10^5 MC38 CEA cells, and 3H1 was started 3 days after the tumors were injected (because of the very aggressive nature of these tumor cells). Mice were treated by injection with either 3H1 conjugated to keyhole limpet hemocyanin (KLH) or the control anti-idiotype 11D10-KLH conjugate every 4 days at the tumor injection site for six courses of treatment. Initially tumors developed in both groups at the same rate. On completion of the six courses of treatment, tumors of six of nine mice treated with the 3H1 vaccine became necrotic and regressed. In the control group, only one of eight mice showed regression. Although only a small number of animals was used in these preliminary experiments, and vaccination started on day 4 after tumor cell inoculation, the data are interesting and the experiments will be repeated. Splenic T lymphocytes isolated from the mice whose tumors regressed showed preferential lysis of MC38CEA cells, but not the parental MC38 cells, by standard chromium-release assay (unpublished data). These studies suggested the therapeutic potential of 3H1 as a tumor vaccine.

Phase 1b clinical trial of patients with advanced colorectal carcinoma (CRC) with anti-Id-3H1

We have completed a phase 1b clinical trial in 23 advanced CRC patients. To augment the immunogenicity of anti-Id vaccine, an adjuvant is typically required. Aluminum hydroxide (alum) has been approved by the

United States Food and Drug Administration (FDA) for use as an adjuvant in humans. For this initial trial, 3H1 was precipitated with alum (alugel) and divided into aliquots in pyrogen-free, sterile glass vials. The final product was tested for sterility, pyrogenicity and general safety tests in guinea pigs before use. An Investigational New Drug Application was approved through the FDA for 3H1. All of the patients had CEA-positive advanced colorectal carcinoma and failed standard therapies. They had been off prior therapy for at least 4 weeks, and staging was repeated 1 month after the fourth immunization and then every 3 months. Patients were treated intracutaneously with either 1, 2 or 4 mg aluminum-hydroxide-precipitated 3H1 every week for four injections. If there was no tumor progression at the end of the four injections, they were then continued on a monthly basis and patients were evaluated every 3 months. Patients were removed from the study if their tumor progressed.

The objective of this phase 1b study was to determine the effects of anti-Id 3H1 on various components of the immune response (both humoral and cellular), to determine the optimum immunomodulatory dose and toxicity of 3H1, and to monitor for clinical responses.

Immune responses to anti-idiotype vaccine

The development of humoral immunity induced by immunization with aluminum-hydroxide-precipitated 3H1 was assessed by testing serum obtained from patients before therapy and after each 3H1 vaccination. Hyperimmune sera from 17 of 23 patients demonstrated an anti-anti-idiotypic Ab3 response as determined by the inhibition of Ab1 (8019) binding to Ab2 (3H1) by serial dilution of patient sera. Of the 17 patients who had an anti-anti-idiotypic response, 13 also had true anti-CEA responses (Ab1′). All of the antibody responses were polyclonal, primarily IgG, and sera from 11 patients mediated in vitro antibody-dependent cellular cytotoxicity (ADCC). Interestingly, samples from patients whose sera mediated in vitro ADCC always contained anti-CEA antibodies. None of the patients in the study had a pre-existing antibody to CEA. We tested for competition between Ab1 and patients' Ab3 for binding to LS174-T cells. If Ab3 has a similar binding site to that for Ab1, it should compete with Ab1 for binding to CEA on LS174-T cells. A fixed amount of radiolabeled 8019 was co-incubated with different concentrations of patients' purified Ab3 or Ab1 preparations and LS174-T cells. Overall, the inhibition curves obtained with Ab1 and Ab3 were very similar at different dilutions. This indicated that the patients' Ab3 bound to the same antigenic epitope as Ab1 and therefore contained antibody molecules with Ab1′ properties. In addition, immune sera from patients bound to live CEA-positive colon carcinoma cell lines and MC38CEA cells, but not to CEA-negative cells lines or MC38 cells, and showed an identical reactivity pattern to that of Ab1 on colon carcinoma specimens by immunoperoxidase staining. Ten patients had idiotypic T cell responses, and 5 had specific T cell responses to CEA.

Time to progression and survival

None of the patients had objective clinical responses to the 3H1 vaccine. However, many patients continued on therapy for 3–21 months and stopped at the time of tumor progression. The median survival for all 23 patients was 11.3 months (95% CI, 7.8–13.7 months) with a 44% 1-year survival (95% CI, 28%–64%). We compared the time to progression and survival in patients who had responded immunologically to 3H1 to patients who were non-responders. Patients who responded survived significantly longer.

Toxicity

Toxicity was typically minimal, only local reactions at the injection site with mild erythema and induration. A few patients developed large, local reactions with swelling that resolved within a few days. Mild fever and chills relieved by acetaminophen occurred in only a few patients. The anti-idiotypic treatment did not have any deleterious effects on hematopoietic cells, or renal or hepatic function. There was no clinical or laboratory evidence of serum sickness. Patients developed human anti-(mouse I_g) antibody (HAMA) since 3H1 was injected as the intact immunoglobulin. However, HAMA has never been a problem in our active immunization protocol.

In summary, we have demonstrated specific active immunity to CEA in over 80% of patients with advanced colorectal cancer treated with an anti-idiotype antibody that "mimics" CEA. To our knowledge, this is the first clinical trial reported in the world's literature demonstrating the ability to generate specific and reproducible immunity to CEA in patients with CEA-positive malignancies [15, 16]. In this phase 1b clinical trial, we could only accrue patients who failed conventional therapy. All of them had widespread advanced disease. The main purpose of this clinical trial was not to assess tumor response, but to determine the host's immunological response to the vaccine therapy. Some primary questions have been resolved. This anti-idiotype antibody can evoke an Ab3 as well as cellular immune response in patients, and any Ab3 so derived, behaves as an Ab1-like antibody (Ab1′). The intensity of the Ab3 response appeared to correlate positively with anti-CEA antibody (Ab1′) and T-cell-proliferative responses. The level of immune response correlated directly with time to progression and survival. Also, the immune response appeared independent of the level of circulating CEA. Patients were able to generate immunity at each of the three dose levels. However, the 2-mg dose was found to be optimal. Toxicity was restricted to local cutaneous

reactions lasting 24–48 h with mild fever and chills and was relieved by acetaminophen.

Next we focused on post-surgical adjuvant patients where the goal was elimination of minimal residual disease (Dukes B, C and resected D). We needed to address the question whether patients on 5-fluorouracil (5-FU) with levamisole or leucovorin generate an immune response to 3H1. We entered 32 patients in the adjuvant setting [18]. All 32 patients entered onto this trial generated high-titer immunoglobulin G and T-cell-proliferative immune responses against CEA. The 5-FU regimens did not have a qualitative or quantitative effect on the immune response. Of 15 patients with Dukes' B and C disease, 3 progressed at 19, 24 and 35 months. Seven of 8 patients with completely resected Dukes' D disease remained on study for 12–33 months; 1 patient with resected Dukes' D disease relapsed at 9 months. One patient with incompletely resected Dukes' D disease was still on study at 14 months without evidence of progression; 8 experienced disease progression at 6–31 months.

3H1 consistently generated a potent anti-CEA humoral and cellular immune response in all 32 patients entered onto this trial. A number of very high-risk patients continue on study. 5-FU regimens, which are the standard of care for patients with Dukes' C disease, did not affect the immune response. These data warrant a phase III trial for patients with resected colon cancer.

To study the cellular immunity invoked by 3H1 at the molecular level, we have cloned and sequenced the cDNA encoding the variable heavy and light chains of 3H1 and deduced the amino acid sequences of the encoded proteins [10]. For the T cells induced by 3H1 to recognize CEA-positive tumor cells, it is necessary for the amino acid sequence of 3H1 to have linear homology to CEA. To identify any cross-reactive peptides of 3H1 and CEA, we compared the amino acid sequences of 3H1 with those of CEA and found several regions of homology in the 3H1 heavy- and light-chain variable domains, as well as in the framework regions. To search for potential cross-reactive T cell epitopes, a number of peptides based on 3H1/CEA homology were synthesized and were used as stimulants in cell proliferation assays, using peripheral blood mononuclear cells from the group of 3H1-immunized CEA-positive cancer patients in the adjuvant setting [18].

Two partially homologous peptides, designated LCD-2 (from 3H1) and CEA-B (from CEA), were identified that generated strong proliferation responses in 10 of 21 patients (stimulation index, 3- to 50-fold), and were extensively studied in 5 of these individuals over an extended period of time (12–24 months).

Analysis of the subtype of the responding T cells demonstrated that primarily $CD4^+$ T cells were stimulated by both 3H1 and these peptides. Two subsets of $CD4^+$ T helper cells have been identified in the literature that produce distinct sets of cytokines. The Th1 subset secretes interleukin-2 (IL-2) and interferon γ (IFNγ), whereas the Th2 subtype secretes IL-4, IL-5 and IL-10. To determine whether the stimulated $CD4^+$ cells constitute predominantly Th1 or Th2 helper cells, the levels of IL-2, IL-4 and IFNγ were assayed in the culture medium from in vitro stimulated bulk peripheral blood mononuclear cells (PBMC) isolated from four 3H1-immunized patients. IL-2 and IL-4 were not detected in significant amounts in the PBMC medium from any of these patients by this assay. However, significant levels of IFNγ were secreted by the PBMC from these four 3H1-treated patients after stimulation with 3H1 and LCD-2, as well as CEA. These data suggest that $CD4^+$ T cells induced by 3H1 were likely Th1. Secretion of both IL-2 and IFNγ from PBMC of CRC patients treated with an anti-Id mimicking the antigen GA733–2 has been reported [13]. In another study, administration of a polyclonal anti-Id mimicking GA733 in gastrointestinal cancer patients also induced $CD4^+$, MHC-class-II-dependent T cells [45]. Traditionally, $CD4^+$ T cells function as helper cells for antibody production. However, these cells have been also shown to have cytolytic functions inducing apoptotic and necrotic cell death [35]. Therefore, T cells primed in vivo by 3H1 therapy have the potential for cytolytic activity against CEA-positive tumor cells. Alternatively, 3H1 vaccination may prime Th1-type helper cells, which, in turn, may induce cytotoxic T cell proliferation by secretion of cytokines such as IFNγ and IL-2.

Anti-idiotype vaccine for breast cancer

We have completed a phase Ib clinical trial for patients with advanced breast cancer with an anti-Id antibody, designated 11D10, which mimics a human milk fat globule (HMGF) membrane epitope. This 11D10 (Ab2) was raised against the anti-HMFG mAb MC-10 (Ab1) [6]. Patients were randomized to a 1-, 2-, 4- or 8-mg dose of 11D10, precipitated with aluminum hydroxide, given intracutaneously four times every other week, then monthly until disease progression. We have treated a total of 33 patients, of which all but 1 had progressive disease or died, with only 19 patients receiving more than four immunizations. Out of these 19 patients, 16 (or 16/33 total patients) demonstrated an anti-anti-idiotypic Ab3 response that inhibited the binding of Ab2 to Ab1 and vice versa. Patients' Ab3 also bound specifically to the purified HMFG antigen. Peripheral blood mononuclear cells from 8 immunized patients showed in vitro idiotype-specific T cell proliferative responses. The results suggested that anti-Id 11D10 can induce both humoral and cellular immune responses in some advanced breast cancer patients who had been heavily pretreated with chemotherapy and radiation, and some had had autotransplants. Toxicity was minimal with only mild erythema and induration at the injection site.

We have also initiated a trial for patients in the post-surgical adjuvant breast cancer setting, randomizing them to the 11D10 vaccine alum-precipitated compared to 11D10 mixed with the QS-21 adjuvant. Of the first 12 patients, 11 have generated an Ab3 response and

purified Ab3 reacted by immunoperoxidase staining with HMFG-positive tumor specimens. All 11 generated idiotype-specific T cell responses. In conclusion, we have demonstrated antigen-specific humoral immune responses and idiotype-specific T cell responses in the majority of adjuvant breast cancer patients [32].

Anti-idiotype vaccine for melanoma

Disialoganglioside GD2 is expressed at high density on melanoma cells. Triggering an active immune response against GD2 with the use of an anti-Id mAb (Ab2) that is the internal image of GD2 offers a novel approach to the treatment of melanoma. We have generated and characterized an anti-Id mAb, designated 1A7, that mimics GD2 in biological and serological assays. 1A7 was raised against an anti-GD2 mAb, 14G2a (Ab1). We have initiated a phase 1b clinical trial for advanced melanoma patients. The primary goals of this trial were to determine immune responses and toxicity to the anti-idiotype vaccine and secondary goals were clinical responses and survival.

A total of 47 patients with advanced melanoma received either 1-, 2-, 4- or 8-mg doses of 1A7 mixed with 100 μg QS-21 adjuvant subcutaneously weekly for 4 weeks then monthly until disease progression. Their median age was 57 years, 32 were male and 15 female, 43% of patients had received prior therapy for metastatic disease, in 55% their disease was confined to soft tissue and 45% had visceral metastasis.

Hyperimmune sera from 40 of 47 patients revealed an anti-anti-Id (Ab3) response, as demonstrated by the inhibition of Ab2 binding to Ab1 and inhibition of Ab1 binding to GD2-positive cells. The 7 patients who did not respond immunologically were those who rapidly progressed and were removed from study prior to their fifth injection with 1A7. There was no qualitative or quantitative difference in immune response among the four dose levels studied. Patient Ab3 was truly Ab1′ since it specifically bound purified disialoganglioside GD2 as well as GD2-positive cells by immune flow cytometry. The isotypic specificity of the Ab3 antibody consisted of predominantly IgG with only minimal IgM. All of the IgG subclasses were represented, with IgG1 the most abundant. One patient has a complete response to 1A7 that has persisted for 24 months. Twelve patients have been stable on the study for 14+ to 37+ months (median 18+ months). Disease progression occurred in 32 patients on the study for 1–17 months (median 5.5 months) and 21 have died after 1–16 months (median 6 months). The Kaplan-Meier-derived overall survival has not been reached but is at least 16 months. For the 26 patients with soft-tissue disease only, the median survival has not been reached. For 21 patients with visceral metastasis, the median survival was 13 months. Toxicity consisted of a local reaction at the site of the injection and mild fever and chills. There was no additional toxicity, such as abdominal pain, which has been seen previously with infusion of murine monoclonal anti-GD2 antibody 14G2a [20].

1A7 has minimal toxicity and generates robust and specific IgG immune responses against GD2 [17, 19]. Objective responses were minimal, but there may be a favorable impact on disease progression and survival that will require prospective randomized trials.

Discussion

There is a renewed interest in the potential of immunological approaches to cancer therapy. It is, therefore, of considerable interest and importance to discuss the relevance of various vaccine-based approaches. Anti-Id vaccines represent an elegant way to generate targeted antigen immunity. The anti-Id approach is less likely to induce autoimmunity if the antigen epitope of interest is not expressed on normal tissues. One of the major problems of human cancer therapy is "immune tolerance", which can be more easily overcome by an appropriate anti-Id vaccine than by a typical multivalent vaccine consisting of whole cells, lysates or antigen-rich supernatant. As an example, patients with CEA-positive tumors are immunologically "tolerant" to CEA. Several laboratories are involved in the design of CEA-based vaccines for cancer patients [12, 49].

A recombinant vaccinia virus expressing CEA has been used as a tumor vaccine [49]. This study demonstrated that CEA can be processed endogenously by human tumor cells. A specific CEA peptide (CAP-1), which is processed by the tumor cells, can be presented by the MHC class I molecule, HLA-A2, to generate cytotoxic T cells that are specific for CEA-positive colorectal cancer cells. In another study, plasmid cDNA encoding CEA was used as a vaccine for the therapy of colorectal cancer patients [12]. However, immune responses generated in cancer patients have been very limited and modest in these studies. One group [42] immunized 18 colorectal carcinoma patients with recombinant CEA plus granulocyte/macrophage-colony-stimulating factor to produce anti-CEA immunity. Interestingly the immune responses generated in patients were mostly against recombinant CEA and not against native CEA on tumor cells. We have used an anti-Id vaccine, 3H1, which mimics CEA to treat colon cancer patients. All of the 32 adjuvant patients generated high-titer, specific IgG anti-CEA immune responses as well as highly specific Th1 helper T cell responses. Another example is breast cancer patients who were treated with vaccines consisting of MUC-1 peptides of different sizes in combination with different potent adjuvants, resulting in mostly anti-MUC1 immunity, which recognized MUC-1 peptides but not MUC1-positive tumor cells [1].

We used an anti-Id, 11D10, which mimics HMFG for breast cancer patients. HMFG and MUC-1 share a similar amino acid sequence. Since MUC-1 epitopes are presumably conformation-dependent, with the use of an anti-Id vaccine we could generate potent anti-HMFG

responses in breast cancer patients that reacted with tumor cells.

Using an anti-Id vaccine to GD2 produced more promising results than did purified gangliosides. There have been a number of vaccine studies targeted to gangliosides [27]. One limitation of vaccination with gangliosides has been the requirement to link the ganglioside covalently to keyhole limpet hemocyanin mixed with a potent adjuvant to produce more potent IgM and, in limited, cases IgG responses [28]. Another limitation of gangliosides is their expensive and difficult purification process. Again, using 1A7, which mimics disialoganglioside GD2, we have been able to generate a consistent high-titer IgG immune response in melanoma patients that is highly specific for GD2.

It was interesting that our anti-idiotype antibody vaccines were effective in eliciting immune responses despite the absence of a strong adjuvant. Aluminum hydroxide precipitation, although considered weakly immunogenic, appeared to be quite adequate in eliciting immune responses. Aggregation of soluble idiotypic determinants by aluminum hydroxide precipitation likely helped to increase antigenicity. Also, our antibody was a foreign protein and was injected as an intact immunoglobulin. The Fc portion of the murine immunoglobulin probably served as a "carrier" to help promote the immune responses.

In the previous "point" article, Dr. Herlyn and her co-workers were unable to induce tumor-protective immunity in mice with an anti-Id raised against GA-733 Ag, whereas they were successful when the Ag was expressed in a viral vector. One reason could be that they did not couple their anti-Id to KLH. In our experience, conjugation of anti-Id to KLH in combination with a strong adjuvant was necessary to raise optimal immunity in mice. Interestingly, as we moved to higher species, such as rabbits, KLH coupling was not necessary; only a strong adjuvant was needed, whereas in monkeys and humans we could use anti-Id vaccines with a weak adjuvant such as alum. Alternatively, the anti-Id antibody generated by Herlyn et al. [29] (and the preceding article in this issue), while meeting the criteria of an internal image antigen, was not potent enough to induce tumor-protective immunity. There are a number of examples in the literature. In one study a number of anti-Id antibody hybridomas were generated against a monoclonal antibody to the L1210 tumor [35]. These anti-Id mAb induced tumor-specific delayed-type hypersensitivity, tumor growth inhibition and T cells that were killer or helper cells. However, only one out of seven anti-Id was able to induce protective immunity in mice against tumor challenge [35]. The greatest challenge in immunotherapy by means of anti-Id antibodies is to identify the right network Ag for a TAA system.

The issue of single-epitope antigen vaccines versus multiple-epitope antigen vaccines remains widely debated. There continues to be a great deal of interest in single-antigen-targeted therapeutic studies in humans. For example, mAb 171 A is being studied in a phase III randomized trial in patients with colorectal cancer [39] on the basis of a small randomized trial that demonstrated improved survival in Dukes' C colorectal cancer patients. Two FDA-approved mAb reagents for cancer therapy, Herceptin and Rituximab are directed against single epitopes. However, we agree that multivalent vaccines targeting distinct epitopes of different tumor-specific antigen molecules might be better. Heterogeneity of TAA expression may be addressed by utilizing cocktails of anti-Id vaccine preparations directed against multiple-target antigens collectively expressed by the vast majority of tumor cells. For example, both CEA and HMFG antigen are expressed by most colon, breast, ovary and non-small-cell lung carcinomas, and a combination of 3H1 and 11D10 could be used to treat these patients. Currently we are developing anti-Id against other potential TAA such as HER2/Neu, prostate-specific membrane antigen and epidermal growth factor receptor, so that a cocktail of anti-Id could be made to treat a variety of cancer patients.

Our data indicate that the anti-Id vaccine approach may have an important role in the treatment of a variety of human cancers. We have observed patients with long-lasting IgG humoral and cellular immune responses to a variety of TAA including CEA, HMFG, GD2 and a highly restricted T cell antigen.

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