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Vaccine Prevention of Cancer: Can Endogenous Antigens be Targeted?

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Abstract

This perspective on the report by Beatty et al. in this issue of the journal (beginning on page **XX**) discusses the prevention of cancer through vaccination strategies that target antigens associated with tumor promotion and progression. Such approaches were first developed for treating cancer. We address cancer vaccination in the context of a mouse model of inflammatory bowel disease expressing MUC1, an epithelial mucin aberrantly expressed during chronic inflammation and in colorectal carcinogenesis, and in a broader context that includes the potential of targeting the tumor microenvironment for immunoprevention in humans. Obstacles in developing effective cancer vaccines, including antigen selection, immunoediting, and tumor-mediated immunosuppression, also are discussed.

What a Vaccine Study in Mice Teaches Us

In this issue of the journal, Beatty et al. (1) describe a vaccination strategy targeting the MUC1 antigen in MUC1+/IL10^{-/-} mice. These mice develop colitis similar to human inflammatory bowel disease (IBD) and eventually progress to develop colitis-associated colon cancer (CACC) related to differential expression of the endogenous mucin MUC1. Vaccination against the MUC1 antigen using a 100-mer MUC1 glycopeptide in combination with E6020, an adjuvant used to enhance antigen-specific immune responses, delayed the inflammation associated with the onset of IBD and prevented progression to CACC. The authors suggest that this strategy has a dual mechanism of action in preventing CACC: induction of a MUC1-specific cellular and humoral response and modulation of local and systemic environments. The MUC1 vaccine reduced both the number of neutrophils within the tumor microenvironment and the number of myeloid-derived suppressor cells (MDSCs) in the spleen. These results demonstrate that vaccination strategies targeting MUC1 in high-risk patients in the setting of chronic inflammation, like ulcerative colitis, may prove efficacious. Furthermore, this study indicates the potential benefit of modulating both tumor-specific immunity and the local and systemic environments.

Preventing Cancer: Why Vaccines?

Prevention of disease through immunization has dramatically reduced—or eliminated—disease burdens worldwide, particularly the burdens of many infectious diseases (2). Vaccines prime the immune system, establishing an antigen-specific memory that mitigates infection when exposure occurs and precludes disease occurrence. For example, vaccinations against

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hepatitis B virus (HBV)- and human papilloma virus (HPV)-specific proteins prevent these infections and have reduced the risk of liver and cervical cancer. These approaches are fundamentally different from those that target endogenous antigens. Viral vaccines target factors that elicit malignant transformation, whereas vaccines against endogenous antigens target the malignant cells and also may target normal cells bearing the antigen of interest. Furthermore, U.S. Food-and-Drug-Administration-approved HPV-vaccine approaches that work well in preventing cancer by blocking infection are ineffective once viruses or virally related premalignancies are established. In contrast, tumor-antigen vaccines can work after tumor antigens are expressed during premalignancy or even by cancers (albeit in the adjuvant or low-volume setting; discussed below in Vaccine Efficacy). Since relatively few human malignancies result from viral infections, it is appropriate to consider alternative vaccination strategies such as the one proposed by Beatty et al.

Most efforts to develop cancer vaccines have focused on the treatment of established cancers, targeting cancer antigens to elicit antigen-directed immune responses. Many investigators have reasoned that successes in treating advanced disease would then justify vaccines in the adjuvant or prevention setting. However, cancer vaccination strategies employed after the appearance of malignant lesions generally have been unsuccessful in inducing tumor regression and improving survival (3). A possible explanation for this failure is the prominent tumor-derived immunosuppression observed in patients with advanced disease. These considerations warrant the testing of cancer vaccines in the adjuvant setting, where relapse-free and overall survival are the key determinants of efficacy (3-4). In this perspective, however, we primarily focus on cancer vaccination strategies to prevent disease.

A distinct challenge of developing cancer vaccines to either prevent or treat cancer is selecting an appropriate antigen that invokes a specific anti-tumor immune response. Whether or not a vaccine can mount an effective anti-tumor response ultimately depends on the functionality of the immune response in the developing tumor's microenvironment. Aside from therapeutic efficacy, the evoked immune response should carry minimal toxicity and must maintain a state of anti-tumor vigilance to be effective. We discuss these challenges to vaccine development in the setting of IBD, which carries a high-risk for CACC. Before discussing IBD vaccination, however, we will describe successful cancer vaccine strategies directed against cancers with a known viral etiology.

Preventing Cancer by Preventing Infection: HBV and HPV

The only successful cancer-preventive vaccination strategies tested thus far have prevented tumor initiation. Chronic HBV infection is associated with 50%–75% of all cases of hepatocellular carcinoma (5). The success of the HBV vaccine—the first human cancer-prevention vaccine—illustrates how critical antigen identification is in vaccine development. In the mid-1960s, Blumberg and colleagues reported the novel Australia antigen, which they discovered by screening sera from leukemia patients (6) and was subsequently identified as the hepatitis B surface antigen (HBsAg). Administration of recombinant HBsAg induces the production of neutralizing antibodies that prevent HBV infection, cirrhosis, and hepatocellular carcinoma. HBV infection alone cannot transform cells, and evidence suggests that chronic HBV infection becomes carcinogenic through recurrent inflammation, which promotes tumorigenesis.

High-risk HPV serotypes directly cause most cervical cancers, can cause genital warts, and are associated with other genital and oropharyngeal and anal cancers (7). Cervical cancer screening via the Papanicolaou test (Pap smear) has been effective in the early detection of HPV-induced cervical dysplasia before its progression to cervical carcinoma. Pioneering work by a multinational group of investigators isolated L1 major capsid antigens from several high-risk

HPVs and demonstrated that recombinantly expressing these antigens in virus-like particles (VLPs) effectively invokes neutralizing antibodies (8). Development of the HBV and HPV vaccines demonstrates how exogenous antigens can invoke potent immunity by preventing infection and avoiding cancer-initiating events.

Preventing Cancer by Preventing Chronic Inflammation: IBD as a Paradigm

Characterized by inappropriate, chronic immune activation in the intestinal mucosa, IBD is a compelling target for cancer immunoprevention. IBD-associated colitis is directly associated with dysplasia that is considered to be an antecedent of CACC (9). An effective IBD vaccine strategy would limit transformation caused by inflammation and enable specific immune effector responses against transformed cells. In targeting MUC1, Beatty and colleagues exploited both aspects of this strategy since aberrant expression of MUC1 increases inflammation during IBD and MUC1 expression is maintained in lesions following malignant transformation. This vaccine strategy is novel because it concomitantly possesses both prevention and therapy attributes.

Tumor Antigens

Historically, tumor antigens have been classified as tumor-specific or tumor-associated. Classic tumor-specific antigens are expressed only by tumor cells, whereas tumor-associated antigens also may be expressed by normal tissue. Tumor-specific antigens are often generated by mutated proteins (e.g., p53), whereas tumor-associated antigens often are aberrantly expressed endogenous proteins (e.g., ERBB2/HER2/neu) that are otherwise normal. As a consequence of genomic instability in tumor cells, each new mutant gene product represents a putative tumor-specific antigen that may be recognized by the immune system. Several mutated genes in the “genomic landscape” of cancer contribute to the transformed phenotype of tumor cells, reinforcing their potential desirability as therapeutic targets, whereas other such genes have less-apparent roles in tumorigenesis (10). Epigenetic instability can unveil antigens that were previously inactive (e.g., the oncofetal antigen alphafetoprotein [AFP]) or minimally expressed in normal tissues (e.g., melanoma antigen [MAGE], a cancer/testis antigen) (11). Gene products with differential overexpression (e.g., HER2) or post-translational modifications (e.g., MUC1) may comprise abundant tumor-associated antigens that, although genotypically wild-type, can invoke an immune response (12).

As potential tumor antigens are discovered, efforts to prioritize these antigens for developing cancer vaccines are critical to focus and accelerate advances in cancer prevention (13). Independent of localized expression pattern, mutant genotype, or contribution to transformed phenotype, useful tumor antigens must be processed and then presented to the host immune system by an antigen-presenting cell (APC), such as a dendritic cell (DC), in order to invoke an antigen-directed immune response. Without this requisite immunogenicity, a tumor antigen may be overlooked by the immune system and therefore may never induce host-protective immune responses through vaccination. Few putative tumor antigens have been shown to be intrinsically immunogenic based on their ability to induce endogenous cellular (14) or humoral responses (15), which have had varying associations with clinical outcomes. A mutant cyclin-dependent kinase 4 (CDK4) antigen in melanoma was recognized by autologous cytotoxic T-lymphocytes (CTLs), which are critical for anti-tumor immunity (16). Endogenous anti-HER2 antibodies have been shown to suppress downstream signaling in HER2-expressing tumor cells (17). These examples of natural, endogenous anti-tumor responses have not been shown to suppress tumor progression or enhance overall survival. They do, however, suggest that enhancing immunity toward tumor antigens by cancer vaccination represents a promising strategy of cancer immunoprevention or immunotherapy.

MUC1

MUC1 is a compelling tumor antigen. It is specifically overexpressed and hypoglycosylated in colonic adenomatous polyps and IBD, which is associated with inflammation, tumor promotion, and progression to CACC. Vaccination with MUC1 peptide has been shown to flatten existing polyps and reduce the number of large polyps in transgenic mice expressing human MUC1 (18). Chronic inflammation and numerous adenocarcinomas induce endogenous anti-MUC1 antibodies, which have been associated with survival benefits in cancer patients (15). These findings were the rationale for the assessment of a MUC1-based vaccination strategy in the high-risk setting of IBD by Beatty et al.

Immunoediting

Mutations, genomic instability, and epigenetic modifications provide potential tumor antigens for therapeutic targeting. These same mechanisms, however, also facilitate tumor escape from immune recognition and elimination, and thus present formidable obstacles to effective vaccination strategies.

First proposed by Burnet and Thomas, the cancer immunosurveillance hypothesis states that tumor cells can be recognized and eliminated by the immune system before the manifestation of clinical symptoms (19-20). Accordingly, it is reasonable to speculate that cancer vaccines might be useful throughout the spectrum of cancer development. Recent sophisticated studies in murine models provided much-needed support for this hypothesis, revealing the critical roles of interferon gamma (IFN- γ) and lymphoid populations in cancer immunosurveillance. However, IFN- γ and lymphoid cells were also shown to alter the tumor itself by decreasing immunogenicity and facilitating growth in immunocompetent animals (21). In an effort to incorporate the tumor-modulating effects of the immune system into the original immunosurveillance hypothesis, Schreiber and colleagues introduced the concept of cancer immunoediting, which encompasses the processes of elimination, equilibrium, and escape (20). During the elimination phase, which corresponds to immunosurveillance, innate lymphoid cells recognize and eliminate accumulated transformed cells. This protective response exerts a selective pressure on genetically unstable transformed cells, allowing them to evolve and persist in a dynamic equilibrium with the immune response (20). The equilibrium phase may last for many years until transformed cells acquire the ability to evade and escape the immune response and manifest as clinically evident disease (20).

How do transformed cells reach the equilibrium and escape phases? A growing body of evidence suggests that genomic instability provides tumors with the ability to produce immunomodulatory factors that can inhibit immunosurveillance. One such factor, transforming growth factor-beta (TGF- β), is produced by many solid tumors and has profound immunosuppressive activity, including the inhibition of DC maturation and T cell proliferation and activation. TGF- β also promotes the accumulation of T regulatory cells (Tregs) that serve to further suppress cellular immunity. Tregs play an important role in suppressing the immune response to endogenous antigens, and the accumulation of Tregs in the tumor microenvironment is associated with poor clinical outcomes (22). Depletion of Tregs in conjunction with cytokine therapy has been shown to increase anti-tumor immunity by enhancing the expansion of tumor-infiltrating CTLs (23). Other soluble mediators, such as interleukin 10 (IL-10), may also suppress effective anti-tumor cellular immunity. IL-10 can activate signal transducer and activator of transcription 3 (STAT3), resulting in the accumulation of immature DCs and Tregs in the tumor microenvironment (24). STAT3 is constitutively expressed by many solid tumors and results in the production of IL-10 and TGF- β , thus further inhibiting anti-tumor immunity (24). Although STAT3 inhibition is associated with tumor regression and enhanced anti-tumor immune responses (25-27), the overall effect

of IL-10 on anti-tumor immunity remains to be determined since several groups have reported that IL-10 can be beneficial to the anti-tumor immune response (28).

Tumor escape also may be facilitated by activation of the tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO). In the setting of chronic inflammation, a subset of DCs upregulates IDO expression. This action promotes the differentiation of naïve cluster of differentiation 4 (CD4)+ T cells into Tregs and enhances the immunosuppressive activity of mature Tregs (29). Therefore, targeting tumor-derived immunosuppression may synergize with vaccination strategies to unleash and amplify the host's anti-tumor immune response.

Vaccine Efficacy

Cancer vaccines have shown efficacy in preclinical models and some promise in clinical trials involving patients with established malignancies. For example, an investigational vaccine, sipuleucel-T, produced a survival benefit in men with rising prostate-specific antigen (PSA) values following definitive local therapy for prostate cancer (30). Sipuleucel-T consists of APCs and other autologous peripheral mononuclear cells that have been exposed *ex vivo* to PA2402, a recombinant fusion protein composed of prostatic acid phosphatase (PAP; an antigen expressed in prostate adenocarcinoma) and granulocyte-macrophage colony-stimulating factor (GM-CSF), an immune-cell activator (30-31). Also, a recent phase III trial of an idiotypic vaccine suppressed follicular lymphoma in the minimal residual disease state (32). Despite these encouraging clinical results, many randomized studies of vaccines in patients with other cancers have failed to demonstrate clinical benefits (33). However, since vaccines are more likely to interfere with cancer development than to cause regression of established cancers that are well defended against immune manipulation, failures in the latter scenario may not reflect their potential for cancer prevention.

Toxicities of Immunotherapy

Attempts to harness and enhance host immune responses can cause autoimmune toxicities. For example, treatment with antibodies directed against CTL antigen 4 (CTLA-4) facilitates T cell activation by blocking an inhibitory receptor on T cells, causing severe enterocolitis, dermatitis, and/or hypophysitis in some patients (34). However, tumor antigen-directed vaccines are more commonly associated with minor toxicities such as local erythema and flu-like symptoms. Therefore, if ultimately effective, these vaccines may be more acceptable than certain other effective preventive agents have been. For example, acceptance of the selective estrogen-receptor modulator tamoxifen has been limited by its association with an increased risk of endometrial cancer (35). On the other hand, higher-risk patients may accept a relatively higher risk of toxicity associated with preventive therapy.

Role of the Tumor Microenvironment

Developing cancers depend on the local environment for growth and survival. Therefore, the interactions between tumors and their microenvironments have been attractive targets for cancer prevention. Stromal cells are the predominant sources of cyclooxygenase-2 (COX-2) in mouse and human intestinal adenomas (36). For example, mucins secreted by neoplastic colonic cells play a critical role in the initial induction of COX-2 in the tumor microenvironment, where COX-2 is an important preventive target, including in inflammatory cells, vascular endothelial cells, and fibroblasts (37). Also, the expression of COX-2 is often increased in colon adenomas and adenocarcinomas (38), and inhibition of COX-2 has been shown to decrease the formation of adenomatous polyps, suggesting a role for COX-2 inhibition in colon cancer chemoprevention (39,40). Cardiovascular toxicities, however, have precluded the widespread use of COX-2 inhibitors in this setting. A MUC1-based vaccination approach may prevent adenomatous polyps and colon cancer, in part, by suppressing COX-2

induction in the tumor microenvironment, avoiding COX-2-inhibitor-related cardiovascular toxicity. It also may be that a MUC1 vaccine combined with low doses of COX-2 inhibitors would be preventive in this setting and potentially could attenuate the cardiovascular toxicity of COX-2 inhibitors.

The tumor microenvironment has other potential targets for immunoprevention. Reactive fibroblasts in the tumor stroma are distinct in expressing fibroblast activation protein (FAP), a surface glycoprotein displaying both dipeptidyl peptidase and collagenase activity. The forced overexpression of murine FAP promotes tumor growth in an animal model (41). DC-based vaccination strategies against FAP have suppressed the growth of carcinomas, adenomas, and lymphomas in preclinical models (42), and a humanized monoclonal antibody against FAP, sibroutzumab, has been tested in early clinical trials in patients with FAP-positive cancer (43). Along with FAP, matrix metalloproteinases (MMPs) contribute to extracellular matrix degradation, which facilitates tumor invasion and neovascularization. Of interest, MMP-9 is highly expressed in MDSCs, and inhibition of MMP-9 abrogates the pro-tumorigenic properties of these cells (44). Direct targeting of angiogenesis also may contribute to effective immunoprevention. Carbone and colleagues showed that pathological levels of pro-angiogenic vascular endothelial growth factor (VEGF), which is produced by many solid tumors, inhibit T cell development and contribute to tumor-derived immunosuppression (45). Therefore, the interplay between the tumor and its environment must be considered when developing a vaccination strategy for cancer prevention.

Questions and Conclusions

While advancing our understanding of effective immunoprevention, the current study also raises interesting questions and avenues for further research. For example, can immunity to other antigens besides MUC1 be generated with results similar to those of Beatty et al., or is MUC1 unique in this regard? If indeed MUC1 can generate protective immune responses in IBD and in colorectal carcinogenesis, what properties of the antigen confer this ability and how can antigens in vaccines be modified to elicit effective cancer immunity? What are the relative potential contributions of tumor-specific immunity and of modulation of the local and systemic environments to cancer prevention? For example, it may be sufficient to limit the accumulation of pro-tumorigenic neutrophils at the tumor site or MDSCs in the periphery to prevent progression to cancer. How safe is cancer vaccination? Whenever immunity to an endogenous antigen is generated, there is a concern of developing autoimmune toxicity. Preventive cancer vaccines should elicit a sustained tumor antigen-specific immune response after only one or a few relatively closely spaced doses, which should help alleviate potential toxicity. In contrast, long-term daily dosing generally required with other chemopreventive agents increases the risk of serious toxicity, such as adverse cardiovascular effects associated with COX-2 inhibition.

All these concerns notwithstanding, it must be remembered that immunoprevention remains a highly effective way of preventing many debilitating diseases. Our understanding of the cancer-relevant immune response and potential targets for intervention is now sufficient to envision broadly applicable approaches for cancer prevention with vaccines directed against endogenous antigens.

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