In recent years, great strides in understanding and regulating the immune system have led to new hope for harnessing its exquisite specificity to destroy cancer cells without affecting normal tissues. This review examines the fundamental immunologic advances and the novel vaccine strategies arising from these advances, as well as the early clinical trials studying new approaches to treat or prevent cancer.
Progress on new vaccine strategies for the immunotherapy and prevention of cancer

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In recent years, great strides in understanding and regulating the immune system have led to new hope for harnessing its exquisite specificity to destroy cancer cells without affecting normal tissues. This review examines the fundamental immunologic advances and the novel vaccine strategies arising from these advances, as well as the early clinical trials studying new approaches to treat or prevent cancer.

Despite multiple approaches to therapy and prevention, cancer remains a major cause of death worldwide. Most nonsurgical approaches targeting rapidly dividing cells, using radiotherapy or chemotherapy, also affect normal cells and result in side effects that limit treatment. In principle, the exquisite specificity of the immune system could be marshaled to precisely target cancer cells without harming normal cells. This hope has motivated much research over several decades but has met with only limited success to date. However, the rapid increase in knowledge of the immune system and its regulation have led to a resurgence of interest in immunologic approaches to target and eliminate cancer (1–7).

A major difference between microbial pathogens and tumors as potential vaccine targets is that cancer cells are derived from the host, and most of their macromolecules are normal self-antigens present in normal cells. To take advantage of the immune system’s specificity, one must find antigens that clearly mark the cancer cells as different from host cells (1, 2), limiting the number of antigens available. Additionally, many potential tumor antigens are not expressed on the surface of tumor cells and thus are inaccessible to antibodies.

The immune system has evolved a solution to this problem: the MHC antigens (HLA molecules in humans) that act as an internal surveillance system to detect foreign or abnormal proteins made inside the cell (Figure 1) (8, 9). A sampling of all proteins synthesized in the cell is cleaved by proteasomes into short fragments (peptides) that are transported into the endoplasmic reticulum. There, the peptides are loaded onto newly synthesized class I MHC molecules, such as HLA-A, -B, and -C. The peptide-MHC complexes are transported to the cell surface for recognition by the CD8+ cells (13). DCs also secrete critical cytokines such as IL-12 and IL-15 that contribute to CTL activation and memory. In addition, a number of regulatory mechanisms that dampen the immune response are exploited by tumors to escape immunosurveillance. These mechanisms include the inhibitory receptor CTLA-4 on the T cells themselves (14) and negative regulatory cells such as the CD25+ regulatory T cell (15–17) and also certain types of CD4+natural killer T (NKT) cells that inhibit tumor immunosurveillance (4, 17–19).

Major hurdles in developing cancer vaccines include: identification of antigens that focus the exquisite specificity of the immune system on cancer cells without harming normal cells; development of methods to induce an immune response sufficient to eradicate the tumor, in the face of self-tolerance to many tumor antigens; and overcoming mechanisms by which tumors evade the host immune response.

Types of tumor antigens
An extensive listing of the known tumor-associated antigens is available, and more are being discovered (20). Tumor antigens can be categorized into four groups: (a) antigens unique to an individual patient’s tumor; (b) antigens common to a histologically similar group of tumors; (c) tissue-differentiation antigens; and (d) ubiquitous antigens expressed by normal and malignant cells. These cat-
To specifically target tumors, antigens must be expressed only in tumor cells. Such unique tumor antigens include mutated proteins, such as mutated oncoprotein ras and mutated tumor suppressor proteins p53 and von Hippel Lindau; and fusion proteins created by chromosomal translocations, such as BCR-ABL in chronic myelocytic leukemia, PAX-FHKR in alveolar rhabdomyosarcoma, and SYT-SSX in synovial sarcoma. K-ras mutations occur in 30–40% of colorectal carcinomas, and mutations of p53 are found in 60–70% of all human cancers. Peptides derived from common ras mutations bind to specific MHC molecules and can generate tumor-specific immune responses. Because the mutations are necessary for generation and maintenance of the neoplastic phenotype, they are expressed by all of the tumor cells and cannot be lost. However, only a short segment of the amino acid sequence encompassing the mutation or fusion breakpoint is actually unique, and this region may not be presented by many common HLA molecules. Another type of tumor antigen unique to cancer cells are antigens with tumor-specific posttranslational modifications, exemplified by MUC1, which shows altered glycosylation in cancer cells, creating neoantigenic sites by exposing protein sequences normally masked by glycosylation.

Cancer vaccine modalities
The vaccine strategies used against cancer depend on how well defined the target antigens are and whether there are conserved antigens that are shared among tumors of the same type in many individuals. We will discuss the rationale for, and experience with, some of the most widely studied approaches (Table 1).

Modified tumor cell vaccines
The richest source of rejection antigens is the tumor itself. However, use of autologous tumor cell vaccines is cumbersome and not amenable to large-scale vaccine production, and tumor samples are often unavailable. Approaches using allogeneic or generic cell lines as vaccines are more widely applicable.
Tumor cells engineered to secrete a number of different cytokines have been shown to protect mice from challenge with the same tumor type (37). Of the cytokines studied, GM-CSF appeared most effective. Local expression of GM-CSF increases DCs and other APCs at the site of injection of vaccination. These acquire, process, and present that peptide.

### Peptide vaccines

Elucidation of the crystal structure of the MHC and of the peptides bound to it (45) and discovery of anchor-residue sequence motifs accounting for binding specificity of peptides to MHC molecules (46) has provided the visual and mechanistic answer to how T cells recognize antigens in the form of short peptides. The observation that short peptide segments (8–10 amino acids) fit into a groove in the MHC molecule, combined with knowledge of the amino acid sequences of tumor epitopes, prompted the use of peptides as therapeutic agents in the treatment of cancer. These observations were followed by cloning of the first human tumor-associated antigen and identification of its nonamer peptide sequence (47). Several strategies have been developed both to improve immunogenicity and to steer the immune system toward desired types of responses. Also, peptides have been loaded onto autologous DCs for administration, as described below. However, individual peptides will be useful only in patients with appropriate HLA molecules capable of presenting that peptide.

### Epitope enhancement

Modification of the amino acid sequence of epitopes, commonly referred to as epitope enhancement, can improve the efficacy of vaccines through several means: (a) increasing affinity of peptide for MHC molecules (4, 48, 49); (b) increasing TCR triggering (32, 33, 50); or (c) inhibiting proteolysis of the peptide by serum peptidases (4, 6, 51). Whenever the peptide sequence is altered, it is important to demonstrate that the T cells induced still recognize the native peptide sequence. There is precedent for epitope-enhanced peptides showing greater efficacy in clinical trials (49) (see below).

### Cytokines, chemokines, and costimulatory molecules as adjuvants

Another approach is to deliver the peptide with adjuvants containing cytokines, chemokines, costimulatory molecules, or other immunomodulators that amplify and direct the immune response (4). Th1 CD4+ T cells and CD8+ cytotoxic T cells crucial for antitumor immunity are induced more efficiently by professional APCs, such as DCs. Based on this knowledge, a recent strategy for the development of peptide vaccines uses a synergistic combination of cytokines that induce DC recruitment (GM-CSF) and costimulatory molecules that induce DC maturation (CD40L or agonistic anti-CD40) (52) in combination with other Th1/CTL-supporting cytokines such as IL-12 and IL-15. Other synergistic combinations have been described in animal models (4). Another adjuvant, a CpG-oligodeoxynucleotide (CpG-ODN), characteristic of bacterial DNA, is used to elicit a broad range of immune cells (53). Differences between humans and mice in the response to cytokines such as GM-CSF and expression of the toll-like receptor 9 (TLR9) for CpG-ODN on DCs will require confirmation during the testing of these approaches in clinical trials. Another approach to target DCs is to use ex vivo–generated DCs that have been preincubated (pulsed) with the peptide of interest. Using peptides with broad MHC class II binding such as pan–HLA-DR–binding peptide (PADRE) (54), endogenous helper epitopes (55, 56), or enhanced helper epitopes (57) to stimulate CD4+ T cell help also results in increased CD8+ T cell responses.

### Clinical trials of peptide vaccines

The best-studied clinical model of peptide vaccination is malignant melanoma. The various immunologic monitoring methods employed are listed in Table 2. Each
has advantages and disadvantages, but none has been shown to provide a surrogate marker for tumor prevention or regression.

Rosenberg and colleagues evaluated vaccination with native gp100 peptide 209–217 and found that it produced only low levels of T cell reactivity in two of eight melanoma patients analyzed, whereas an epitope-enhanced gp100 (g209-2M) peptide generated strong T cell reactivity in 10 of 11 patients immunized (49). Nevertheless, only a single objective clinical response was reported. Immunization with g209-2M, combined with high dose IL-2 treatment, produced anti-tumor responses in 42% of patients, although T cell reactivity was observed in less than 10% of patients. In the adjuvant setting, Smith et al. (58) found a vaccination frequency of every two or three weeks resulted in a median frequency of CD8+ cells binding g209-2M tetramers of 0.34% after 6 months, compared with 0.02% before vaccination, whereas less frequent vaccination gave substantially lower responses of 0.03%. Addition of either IL-12 (59) or GM-CSF (60) increased this percentage slightly. The impact of age was striking (58). In patients under 60 years old, the median number of tetramer-positive CD8+ cells induced by vaccination was 0.64%, whereas in those over 60 it was 0.08%.

Immunization with tyrosinase peptide has been significantly less effective despite epitope enhancement. Immunization with the tyrosinase 370D peptide in incomplete Freund’s adjuvant (IFA) with or without cytokines, including IL-12 (59) or GM-CSF (60), only rarely resulted in low numbers of tetramer-positive CD8 cells or cytokine production. The levels of 370D-responsive cells detected by enzyme-linked immunosorbent spot (ELISPOT) assay have been low (0.01–0.03% of input cells) (61). The use of

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole tumor cell</td>
<td>1. Studied extensively</td>
<td>1. Requires availability of autologous tumor or an allogeneic cell line sharing the relevant tumor antigens;</td>
</tr>
<tr>
<td></td>
<td>2. Can be processed to enhance antigen presentation (e.g., irradiated tumor cells or tumor lysates);</td>
<td>2. Poor ability to stimulate immune responses;</td>
</tr>
<tr>
<td></td>
<td>3. Can be administered with adjuvants (e.g., BCG, KLH, viruses, etc.);</td>
<td>3. Few responses and little benefit reported when used adjuvantly in randomized clinical trials</td>
</tr>
<tr>
<td></td>
<td>4. Likely to express the relevant tumor antigens;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Antigens need not be defined</td>
<td></td>
</tr>
<tr>
<td>Gene-modified tumor cells</td>
<td>1. Likely to express the relevant tumor antigens;</td>
<td>1. Requires availability of autologous tumor or an allogeneic cell line expressing the relevant tumor antigens;</td>
</tr>
<tr>
<td></td>
<td>2. Antigens need not be defined</td>
<td>2. Weak antigen presentation by many tumors;</td>
</tr>
<tr>
<td></td>
<td>3. Often engineered to coexpress immunostimulatory molecules and cytokines (e.g., GM-CSF, IL-2);</td>
<td>3. Long manufacturing time;</td>
</tr>
<tr>
<td></td>
<td>4. Use of allogeneic tumor cell lines and fibroblasts are under investigation as an approach to accelerate vaccine production;</td>
<td>4. Need for ex vivo cell culture;</td>
</tr>
<tr>
<td></td>
<td>5. Some immunological and clinical responses reported</td>
<td>5. Cost, time, and labor intensive</td>
</tr>
<tr>
<td>Plasmid (naked) DNA</td>
<td>1. Constructed to express the relevant tumor antigen;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Easy to produce and stable;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Can be administered as a direct injection or biolistically (“gene gun”)</td>
<td></td>
</tr>
<tr>
<td>Peptides</td>
<td>1. Can limit immune response to epitopes distinct from the wild type (e.g., point mutations or breakpoint-fusion genes);</td>
<td>1. Requires knowledge of the specific epitope;</td>
</tr>
<tr>
<td></td>
<td>2. Epitopes can be enhanced;</td>
<td>2. Immunogenicity restricted to a limited number of MHC molecules;</td>
</tr>
<tr>
<td></td>
<td>3. Easy to produce and stable;</td>
<td>3. Usually requires the addition of an adjuvant for immunogenicity</td>
</tr>
<tr>
<td></td>
<td>4. Can be combined as cocktails of peptides;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Some immunological and clinical responses reported</td>
<td></td>
</tr>
<tr>
<td>Viral gene transfer vectors</td>
<td>1. Engineered to express the relevant tumor antigen;</td>
<td>1. Immunodominance of viral antigens over tumor antigens;</td>
</tr>
<tr>
<td></td>
<td>2. Can be engineered to coexpress immunostimulatory molecules and cytokines;</td>
<td>2. Weak antitumor responses seen with most viral vectors;</td>
</tr>
<tr>
<td></td>
<td>3. Wide variety of available vectors (e.g., adenovirus, pox viruses, lentiviruses, etc.);</td>
<td>3. Preexisting immunity against viral vectors may attenuate the antitumor response;</td>
</tr>
<tr>
<td></td>
<td>4. Some cellular immune responses reported</td>
<td>4. Risk of toxicity with “live” viruses</td>
</tr>
<tr>
<td>Antigen-modified DCs</td>
<td>1. Use of powerful APCs;</td>
<td>1. Need for ex vivo cell culture;</td>
</tr>
<tr>
<td></td>
<td>2. Techniques available to generate large numbers of clinical grade DCs;</td>
<td>2. Cost, time, and labor intensive;</td>
</tr>
<tr>
<td></td>
<td>3. Target antigens may be defined or uncharacterized;</td>
<td>3. Optimal technique for antigen loading remains undefined;</td>
</tr>
<tr>
<td></td>
<td>4. Multiple antigen loading techniques (e.g., peptide, lysates, whole protein, RNA transfection, viral vectors, etc.) are available;</td>
<td>4. Possibility of tolerization by immature DCs;</td>
</tr>
<tr>
<td></td>
<td>5. Some immunological and clinical responses reported</td>
<td>5. Lack of criteria for standardization of final product</td>
</tr>
</tbody>
</table>

BCG, bacille Calmette-Guérin; KLH, keyhole limpet hemocyanin.
peptide vaccines may be additionally complicated by the choice of adjuvants. Most studies used IFA, but in the study by Schaed et al. (61), patients showed no response with 370D peptide in IFA, whereas almost 50% of patients showed low-level ELISPOT responses with QS21 or GM-CSF as adjuvants.

The importance of epitope enhancement is supported by the promising results of vaccination with the epitope-enhanced carcinoembryonic antigen (CEA) peptide (33). FLT3 ligand–expanded DCs were pulsed with this peptide to immunize patients with advanced colorectal cancer. Five of 12 patients immunized developed greater than 1% tetramer-positive CD8+ T cells, and two showed clinical responses.

Despite the small sample sizes and the variable populations treated, some principles emerge. Immunization with native peptide sequences is often insufficient to generate reactive T cells and clinical responses in most patients. Epitope-enhanced peptides can generate T cell responses but not always clinical tumor responses. Adjuvants, including cytokines and costimulatory molecules, improve the immunogenicity of peptide vaccination. Paradoxically, combining peptide vaccination with IL-2 significantly reduced detection of specific T cells in blood, but nearly half the patients showed objective cancer regressions (49), possibly due to IL-2–induced innate immunity combined with vaccine immunity. In a new approach, blockade of the negative regulatory molecule CTLA-4 showed promise when combined with vaccination with g209-2M in melanoma patients. Three of 14 patients treated had objective tumor regressions although at the cost of development of autoimmune disease, including bowel, liver, and pituitary dysfunctions (62). Blockade of other negative regulatory pathways has been shown much promise for antitumor vaccination.

DNA vaccines
Intramuscular injections of naked DNA expression plasmids have been shown to generate immune responses (68, 69). Such DNA vaccines introduce tumor antigen genes into DCs for endogenous processing and presentation to CTLs in draining lymph nodes or into other cells for cross-presentation by DCs, without the need for a viral vector (Figure 2). Thus, problems of competition from viral vector epitopes, reduced efficacy due to prior systemic immunity to the viral vector, and potential dangers associated with a live virus are avoided. Constitutive, tissue, or tumor-specific promoters may be used for selective expression.

The results of a number of plasmid DNA vaccine trials have been reported. Among 12 patients with follicular lymphoma vaccinated with plasmids encoding tumor-specific idiotypes (70), four mounted a humoral anti-idiotype or a specific anti-idiotype T cell proliferative response. In another trial, among 17 patients with metastatic colorectal carcinoma vaccinated with a plasmid encoding both CEA and hepatitis B surface antigen (HBs) as a control (71), six developed protective levels of anti-HBs antibody, but none developed antibody to CEA, although 4 of 17 developed a lymphoproliferative response to CEA. Thus, DNA vaccines have not yet shown much promise for antitumor vaccination.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Immune response monitoring for clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay</strong></td>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>Proliferation</td>
<td>Technically simple</td>
</tr>
<tr>
<td>Tetramer staining</td>
<td>Quantitative cell number; subset analysis possible</td>
</tr>
<tr>
<td>Cytokine flow cytometry</td>
<td>Quantitative cell number; functional assay; subset analysis possible</td>
</tr>
<tr>
<td>ELISA</td>
<td>Functional assay</td>
</tr>
<tr>
<td>ELISPOT</td>
<td>Quantitative cell number; functional assay</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>Functional assay; relative cell quantitation</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Universal reagents; indirect measure of function</td>
</tr>
<tr>
<td>Limiting dilution</td>
<td>Quantitative cell number; functional assay</td>
</tr>
</tbody>
</table>
**Dendritic cell vaccines**

DCs are professional APCs and are the most powerful stimulators of naïve T cells (13, 72, 73). Immature DCs sample the antigenic environment through phagocytosis, micropinocytosis, receptor- and lectin-mediated endocytosis and are more effective at processing antigen. When DCs encounter inflammatory mediators such as bacterial LPS, or TNF-α, they mature. Helper T cells also induce DC maturation via CD40 ligand interaction with CD40. As they mature, DCs downregulate their antigen uptake and processing machinery; express CD83; upregulate MHC, costimulatory molecules (CD80 and CD86), and the chemokine receptor CCR7; and travel to lymph nodes where they activate antigen-reactive T cells.

Demonstration of acquired defects in DC maturation and function in tumor-bearing animals and cancer patients suggests a rationale for using ex vivo–generated DCs as antitumor vaccines. Gabrilovich and colleagues reported ineffective CTL induction in a murine mutant p53 fibrosarcoma model associated with defects in DC function (74). Supernatants from tumor cells suppressed DC maturation, ultimately attributed to an effect of VEGF (75). In contrast, DCs generated in vitro from marrow progenitors stimulated allogeneic T cells and induced mutant p53 peptide–specific T cell responses (74, 76, 77). Immunization with mutant p53–peptide–pulsed DCs inhibited the growth of established tumors in the mice.

DCs pulsed with tumor lysates (78), tumor protein extracts (79–81), synthetic peptide tumor epitopes (74, 82), or DCs fused with irradiated tumor cells (83) could generate protective immunity to subsequent tumor challenge. Transfer of nucleic acids encoding tumor antigens into DCs using plasmid transfection (84), retroviral vectors (85), recombinant adenoviruses (86), lentiviruses (87), or electroporation of tumor RNA (88) has been effective. Antigens can also be targeted to DCs by coupling to DC-specific antibodies (88). Transfer of genes encoding costimulatory molecules (B7) and cytokines (IL-12) into DCs has also enhanced antitumor vaccine efficacy (80).

Clinical trials of DC vaccines have depended on development of techniques for obtaining large numbers of clinical grade human DCs (89, 90). Currently, two general approaches are used: (a) purification of immature DC precursors from peripheral blood (72); and (b) ex vivo differentiation of DCs from CD34+ hematopoietic progenitor cells (91, 92) or peripheral blood monocytes (90), commonly by culture of monocytes with GM-CSF and IL-4 (Figure 3). Immature DCs can be matured with CD40 ligand, LPS, or TNF-α (93). In mice, CD40 ligand–mediated maturation was the most effective approach for DC vaccine preparation (94).

In humans, the finding that two healthy volunteers receiving immature DCs pulsed with influenza matrix peptide (FMP) had a reduction in FMP-specific CD8+ T cell activity (95) raised concerns
that immature DCs might induce tolerance to antigens (96, 97). The recent trend has been to use DCs matured using TNF-α, CD40 ligand, monocyte-conditioned media, or cytokine cocktails (98, 99).

Clinical trials of anticancer DC vaccines. A number of DC cancer vaccine trials have been reported. Hsu et al. reported the first DC vaccine trial for treatment of cancer in patients with follicular B cell lymphomas (100), which express a unique clonal B cell receptor (idiotype, Id) that can distinguish lymphoma cells from nonmalignant lymphocytes. Initially, ten patients were treated with peripheral blood DCs pulsed with a tumor-specific Id protein. Eight developed Id-specific cellular proliferative responses, and one developed a specific CTL response. Two patients had complete responses (CRs), one a partial response (PR), and another a complete molecular response. A follow-up study with an additional 25 patients found that 15 of 23 generated T cell and humoral anti-id responses (101).

Multiple myeloma (plasma) cells also express unique clonal immunoglobulin idiotypes (102–104) but do not express Id protein on the cell surface, instead producing large amounts for secretion. Myeloma cells can still be detected by CTLs that recognize Id peptides presented by HLA molecules. Of 26 patients treated with Id-pulsed DCs after high-dose chemotherapy and stem cell transplantation, four developed Id-specific T cell proliferative responses. Although the stem cell transplant itself produced 5 CRs and 21 PRs, 8 patients with PRs had further declines in their serum monoclonal spike after the DC vaccination, and of the four patients that developed an immune response, two remained in CR at 35 and 28 months after transplantation (103). In another trial (104), patients with residual low-volume myeloma after transplant were immunized with DCs pulsed with autologous serum as a source of Id. Of the 13 evaluable patients, three achieved a CR and 3 a PR.

DC vaccines have also been studied in patients with solid tumors. Of 21 patients with recurrent or metastatic prostate cancer and elevated serum prostatic acid phosphatase (PAP) treated with DCs pulsed with rodent PAP, ten developed T cell–proliferative responses to PAP (105). Among 16 patients with metastatic melanoma receiving peptide- or tumor lysate-pulsed DCs injected directly into lymph nodes, eleven developed delayed-type hypersensitivity responses to peptide-pulsed DCs, and two had durable CRs (106). Among 11 melanoma patients receiving monocyte-derived DCs pulsed with HLA-A1–restricted MAGE-3 melanoma peptide, eight developed a CTL response, and some minor tumor regressions were observed (107). Recently, 20 patients with advanced pancreatic, hepatocellular, cholangiocarcinoma, or medullary thyroid carcinoma were treated with monocyte-derived DCs pulsed with tumor lysates, matured with TNF-α, and administered along with daily subcutaneous IL-2 (108). Although 18 of 20 patients developed DTH responses and 8 had a decline in serum tumor markers, no CRs or PRs were seen. A small, uncontrolled clinical trial in metastatic renal cell carcinoma with tumor RNA-loaded DCs reported an unusually low mortality from tumor in the follow-up period (109).

Inconsistency in trial results may relate to several variables, including the type and quality of DCs that vary with method of generation and maturation, the technique used for epitope loading, and the dose, route, and frequency of vaccination. Animal models indicate benefit of multiple vaccinations compared to a single dose (74) and of subcutaneous and intradermal administration (110). Thus, while the use of DCs as a vehicle for therapeutic cancer vaccination holds promise to circumvent some strategies tumors use to evade the immune system, there are still many technical issues to be resolved in this approach, which needs to be compared directly with other vaccine modalities.

Future strategies to enhance cancer vaccine efficacy
It is becoming clear that the immune response may be hindered by the hurdles created by tumors to evade the immune system. As noted, VEGF and other tumor-derived factors can inhibit DC
maturation, and maturation of DCs ex vivo can circumvent this roadblock. We also discussed under “Peptide vaccines” (above) the use of cytokines, chemokines, and costimulatory molecules to activate and direct the immune response toward the appropriate type, as well as epitope enhancement to improve binding of tumor antigen epitopes to MHC molecules or TCRs.

Another area generating much recent interest is the possibility of overcoming mechanisms that downregulate or attenuate the immune response (Figure 4). Such mechanisms may have evolved to reduce inflammation and immunopathology or to prevent autoimmune. Tumors have co-opted these mechanisms to evade immuno-surveillance. T cells themselves express inhibitory receptors, the best studied being CTLA-4 (111, 112). This binds costimulatory molecules CD80 and CD86, but, instead of activating the T cell, dampens its response. Blockade of CTLA-4 has been shown to improve tumor immunosurveillance and amplify effects of cancer vaccines in animals (14, 111). Recently, monoclonal anti–CTLA-4 antibodies have been studied in clinical trials alone or in conjunction with cancer vaccines (62). A substantial number of objective responses were found in a melanoma trial, but not without a number of autoimmune side effects, all reversed when therapy was stopped.

Another regulatory mechanism is the CD25+CD4+ regulatory T cell (15, 17, 113). Such cells are induced by antigens, especially in the presence of high IL-2 levels, but their effector activity is not antigen-specific (113). Blockade or elimination of these cells has been shown to enhance tumor immunosurveillance and efficacy of antitumor vaccines (15). Furthermore, concurrent blockade of CD25+CD4+ regulatory T cells and of CTLA-4 was synergistic in augmenting efficacy of a cancer vaccine (16).

Another novel immunoregulatory cell is the CD4+ NKT cell. These are T lymphocytes that share certain markers with NK cells but also express normal CD3 and αβ TCRs. However, the TCRs used are limited to a few types that predominantly recognize glycolipids presented by a nonclassical class I MHC molecule, CD1d (114). These cells play a role in regulating immune responses causing autoimmune diseases, such as diabetes (115). Recently, we found that CD4+ NKT cells also can inhibit tumor immunosurveillance in a mouse fibrosarcoma model (17–19) and an orthotopic breast cancer model (116), and have now extended these findings to a non-regressor colon cancer model (19) (Park et al., unpublished results) (Figure 4). Elimination of NKT cells, or blockade of their effector mechanisms, such as IL-13 or downstream TGF-β, prevented tumor recurrence (17–19) and improved CTL responses and antiviral efficacy of a peptide AIDS vaccine in mice (52). Whereas regulatory NKT cells express predominantly Th2 cytokines, other NKT cells making IFN-γ can actually contribute to tumor immunosurveillance (17). Factors influencing these opposing roles of NKT cells are under study. Thus, we propose that blockade of NKT cell negative regulatory cytokines can be used as a strategy to increase the efficacy of anticancer vaccines (17).

A complementary approach is to selectively induce high avidity CTLs (4, 117), which were first shown to be more effective at clearing virus infections (118) and then also to be more effective at killing tumor cells and eradicating tumors (119, 120). We recently found that increasing levels of costimulation, using a triad of costimulatory molecules — CD80, ICAM-1 and LFA-3 — in conjunction with a vaccine, can selectively induce CTLs skewed toward higher avidity and more effective at killing tumor cells (67). More-
over, IL-15 expressed by a vaccine has also been found to selectively induce longer-lived CTLs that may be more effective (121) and also a higher average avidity of CTLs (Oh et al., unpublished results). Indeed, we suggest that the recently discussed role of CD4+ T cell help and signal strength in inducing long-lived memory CD8+ CTLs (122–125) may be mediated in part by helper cell stimulation of DCs to produce IL-15 when they present antigen to CTLs, as we have mimicked with IL-15 in the vaccine (121). These strategies may be useful in optimizing anticancer vaccines.

Conclusion

As prophylaxis against acute infectious diseases, vaccines have been shown to protect healthy individuals, and cancer vaccines have achieved widespread success. Increased understanding of immune-mediated tissue destruction and the defects that predispose to cancer have led to the development of tumor vaccines designed to inhibit tumor immunosurveillance and immune responses to anti-tumor vaccines. A number of promising new cancer vaccine strategies have entered clinical trials, and we eagerly await their findings.

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