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Tumor Vaccines for Breast Cancer

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SUMMARY

The goal of cancer vaccines and immunotherapies is to train the immune system to recognize cancer cells and destroy them. Immune responses play a dynamic role in the development of cancers, from immunosurveillance to immune escape; from in situ immune dysregulation to metastatic spread. The systematic identification and targeting of molecules involved in the immune response has led to a wide variety of potential immunotherapeutic targets for the treatment of breast cancer. Extraordinary advances in molecular immunology have led to a detailed understanding of tumor antigens, antigen presentation, innate immunity, cytokine and chemokine pathways, and immunoregulation. Many of these vaccine therapies are already in clinical development. It is the rational and rapid translation of these scientific discoveries into effective therapies for patients with breast cancer that poses the greatest challenge, and opportunity, to realize the potential of tumor vaccine therapy for breast cancer.

CANCER AND THE IMMUNE RESPONSE

The immune system is a complex multi-cellular network, which can quickly accommodate or combat novel pathogens. This network of activating and inhibitory cells and molecules result in a tight balance between immunity and autoimmunity. It is the ability of the immune system to distinguish self from non-self that results in effective clearance of pathogens and immunologic memory. The primary challenge facing the field of tumor immunology is that, unlike infections, all tumor cells contain self-antigens that vary from normal tissue, primarily by mutation or by expression level. Many of these self-antigens are critical for biologic processes, such as DNA replication, or are expressed at some level on normal tissues. Thus, effective tumor immunity carries the risk of clinically significant autoimmunity.

There are several lines of evidence suggesting that breast cancer is subject to immunosurveillance. A case–control study of 176 women with breast cancer showed a genetic association with protective human leukocyte antigen (HLA) class II alleles (1). MHC molecules are down regulated in 20% to 50% of primary breast tumors and cell lines, and class II molecules have been detected in around 30% of breast carcinoma lesions (2, 3), but this is of unclear clinical significance. As with ovarian cancer, melanoma, and colon cancer, lymphocytic infiltrates have been shown to be associated with improved overall survival in breast cancer (4, 5). T-cells recognizing MUC-1 and HER2/neu-derived antigens have been isolated from the blood of breast cancer patients (6, 7). Evidence that T-lymphocytes can effectively target breast cancer tumor cells is demonstrated by the small, but measurable graft-versus-tumor effects that have been shown in patients, undergoing donor-lymphocyte infusion after allogeneic stem-cell transplantation (8–10).

Innate immunity

The identification of the molecular pathways involved in the innate immune response has led to numerous clinical trials of immune adjuvants. The innate immune response represents

the first line of defense against pathogens, and includes natural barriers (skin, mucosa, and the blood–brain barrier), cytokines, complement, and cellular immunity including natural killer cells (NK cells), neutrophils, and macrophages (11). This response is primarily mediated by activation of the family of toll-like receptors (TLRs) on macrophages. There are at least 10 known human TLRs, each of which is stimulated by specific molecular structures. These agonists are potent immunostimulants and include double-stranded RNA (which activates TLR3), lipo-polysaccharide (which activates TLR4), and CpG DNA (which activates TLR9). TLR stimulation leads to the destruction of pathogens by means of activated macrophages or natural killer (NK) cells as well as cytokine release for immune amplification and dendritic cell maturation (12). As a result, TLR agonists are being developed as adjuvants in both infectious and cancer vaccine trials. For example, CpGs are synthetic 8 to 30 base-long oligonucleotides that mimic pathogenic DNA, and activate TLR9 on dendritic cells to augment T-cell responses to vaccination (13, 14).

In addition to TLRs, NKG2D is an activating receptor expressed on NK cells and macrophages. NKG2D can interact with ligands expressed by tumor cells, causing alteration of innate immunity (15). In animal models, NKG2D ligand expression early in tumor development protects the host from tumor initiation (16). These ligands include major histocompatibility complex (MHC) class I chain-related protein A and B (MICA and MICB). MIC proteins are overexpressed in most epithelial cancers, including breast tumors (17, 18), and soluble major histocomputibility complex (MHC) antigens secreted by tumors down regulate T-cell activity (19). In addition, the inhibitory NK cell ligands HLA-E and -F have been detected on a subset of breast tumor cell lines (3), and soluble HLA-G, which induces apoptosis of T-cells that has been detected in malignant ascites (20). The mechanisms used by tumor cells to regulate the innate immune response are all potential targets for therapeutic intervention.

Adaptive immunity

The adaptive immune response, which involves T- and B-lymphocytes, is required for immunologic memory. This response is initially slower than the innate response but leads to rapid and highly specific memory responses on subsequent challenge. Antigens may be either directly presented by tumor cells, or cross-presented by antigen-presenting cells (APCs). Either way, the antigens are degraded to peptide epitopes, which are then bound to MHC molecules, for presentation to T-cells (Fig. 1). MHC class I peptide epitopes that are created by proteasomal cleavage are structurally limited by the size of the MHC peptide-binding groove. Therefore, for a given antigenic sequence, potential MHC class I-binding epitopes, such as the E75 peptide of HER2/neu (21) or the I540 peptide of telomerase (22), can be predicted with some accuracy using algorithms based on the primary sequence of the protein. A limited number of tumor antigenic epitopes have been directly sequenced from purified class I MHC molecules (23, 24), but the low concentration of specific peptides has made direct identification of tumor antigenic sequences by mass spectrometry difficult.

In contrast, MHC class II molecules primarily bind peptides derived from exogenous antigen that is endocytosed by APCs for presentation to CD4+ T-cells. Since the peptide-binding groove of class II molecules is structurally more flexible than that of class I molecules, prediction of antigenic peptides is more difficult and requires systematic empirical identification with the use of overlapping peptide sets. As a result, fewer class II peptide epitopes from tumor antigens have been identified and tested in clinical trials (25, 26).

Antibody immunity

The natural development of B cell antibody responses to tumor antigens is dependent on antigen overexpression, mutation, apoptosis, changes structural, and aberrant glycosylation

(27). Aberrently glycosylated carbohydrate antigens, such as Tn, are expressed by tumor cells and have been used in clinical vaccine trials in breast cancer (28–31) with evidence of immunogenicity. Serologic expression cloning has been used to detect antibodies to multiple breast cancer protein antigens, including HER2/neu, p53, MUC1, and NY-ESO-1 (32–35). In prostate cancer, patterns of autoantibody production correlate with disease outcome (36), suggesting that autoantibodies may be useful as proteomic biomarkers both for diagnosis and prognosis (27). Similarly, antibodies to HER2/neu have been detected in serum samples from 20% of patients with HER2+ early-stage of breast cancer (32). Although HER2 antibody titers of exceeding 1:5000 have been reported, it is unclear whether they confer a protective immune response. Since B-cell immunity often correlates with T-cell immunity, autoantigen identification has led to the identification of T-cell antigens for vaccine development (37, 38). Since tumor antigen-specific antibodies can enhance tumor antigen cross-presentation, combined vaccine and antibody therapy, such as HER2-vaccines and trastuzumab, may augment anti-tumor immunity.

Cytokine dysregulation

In the tumor microenvironment, tumor cells both actively down regulate the immune function and co-opt the immune molecules for tumor activation, invasion, and metastasis (39–41). Molecules such as vascular endothelial growth factor (VEGF), interleukin-6, macrophage-colony stimulating factor (M-CSF), cyclooxygenase 2 (COX-2), interleukin-10, stem cell factor-1, and transforming growth factor (TGF β) are abundant in the tumor microenvironment, resulting in altered dendritic cell and T-cell function (Fig. 2) (39). In addition to secreted molecules, transmembrane molecules such as FasL (CD95L), B7-H1/PD-L1 and B7-H4 are potent inhibitors of T-lymphocyte function (42). FasL (43), B7-H1 (44), and B7-H4 (45) are all expressed by subsets of breast tumors and are potential targets of immune intervention.

Antigen presentation and dendritic cells

Although tumor antigenic peptides can be presented directly from tumor cells, professional APCs, in particular dendritic cells, are essential for priming naïve T-cells and activating the immune response (46). Tumors of epithelial origin generally do not express costimulatory signals, such as B7, CD40, 4-1BBL (47), and OX40L (48) that are required for activation of effective T-cell responses. Immature DC's may actively endocytose necrotic, apoptotic, or antibody-coated tumor cells ("cross-presentation"), and then undergo maturation upon activation of TLRs, CD40 ligand, or cytokine signals such as TNF-alpha (Fig. 1). Upon maturation, dendritic cells upregulate MHC molecules and costimulatory molecules, secrete cytokines and chemokines to enhance the migration of lymphocytes, and express chemokine receptors for migration to lymph nodes (49).

There is mounting evidence that dendritic cells have abnormal function in cancer patients (Fig. 2) (50). Indoleamine 2,3-dioxygenase (IDO) is involved in tryptophan catabolism and is thought to play a role in placental-based maternal immune tolerance. Accumulation of IDO in dendritic cells correlates with impairment of T-cell function in vitro and has been observed in lymph nodes of patients with melanoma and breast cancer, and other tumors (51, 52). IDO accumulation in dendritic cells can predate the development of overt lymphnode metastases. Inhibitors of IDO are now being developed as potential immune adjuvants.

There have been multiple clinical trials of vaccine delivery with the use of dendritic cells. The cells are usually isolated from peripheral blood by means of leukapheresis. They are cultured in vitro with the cytokines GM-CSF and interleukin-4, loaded with antigen, and matured ex vivo to enhance antigen presentation and costimulation of T-cells before being

injected into patients. Antigen may be delivered as peptide, protein, RNA (53), or tumor lysates (54) (Table 1). In addition, dendritic cells have been directly fused with autologous breast cancer tumor cells, which allows for the presentation of multiple tumor antigens (55). Although dendritic cell-based vaccines have had minimal side effects and have induced measurable T-cell immunity, few durable clinical responses have been reported (55–57). However, a dendritic cell-based vaccine was shown to confer a modest survival benefit in hormone-refractory prostate cancer (58). Because dendritic cell production must be performed in specialized clinical laboratories, alternative strategies, including using artificial APCs or targeting antigen directly to dendritic cells in vivo using DC-targeted antibodies, microparticles, electroporation, or nanotechnology are being explored.

TARGETING TUMOR ANTIGENS

Antigen-specific vaccines

The ideal breast cancer vaccine would induce broadly reactive immunity to multiple types of breast cancer without causing clinically significant autoimmunity and, most important, be clinically effective. One approach to minimize autoimmunity and enhance specificity of vaccines is to target them to specific protein antigens that are overexpressed on the tumor cells but that have limited distribution in normal tissue. Many breast cancer tumor antigens are also expressed on tumor cells in other epithelial-derived cancers, such as ovarian cancer and colon cancer, and have been targeted in early-phase clinical trials in breast cancer and other solid tumors. In addition to MUC-1, HER2/neu, and telomerase (see subsequently), target antigens include CEA (59, 60), cyp1B1 (61), survivin (62, 63), and others (Table 1).

MUC-1—Overexpression and aberrant glycosylation of mucin-1 (MUC-1) antigen by epithelial tumors results in endogenous antibody responses in cancer patients to MUC-1 antigen (64). This finding has led to the identification of MUC-1-derived peptide epitopes that induce T-cell responses. MUC-1-based clinical trials have used peptides (65–69), protein (70), pulsed dendritic cells (61), or keyhole limpet hemocyanin (KLH) adjuvant (31).

HER2/neu—The HER2/neu antigen is a well-known target of antibody-mediated immunotherapy in breast cancer. The initial demonstration of multiple HLA-A2-binding peptides derived from the HER2/neu protein has led to multiple clinical vaccine trials. Initial studies using peptide and adjuvant have demonstrated safety with minimal toxicity (61, 71–73), but induced cytotoxic T-cells that failed to lyse tumor cells (21). To augment CD4+ T-cell immunity, HER2-derived class II peptides (26, 74), or the HER2 intracellular domain (75, 76), have been used for vaccination. A recent study of vaccination of high-risk patients in the adjuvant setting showed a trend toward improved disease-free survival in patients who received a HER2 peptide-based vaccine (85.7% vs. 59.8% in unvaccinated patients).

hTERT—The catalytic subunit of telomerase, hTERT, is a widely expressed tumor antigen, present in more than 85% of all human cancers (22). Initial clinical trials of dendritic cells pulsed with hTERT-derived peptides or hTERT RNA resulted in measurable hTERT-specific immunity (78, 79), but hTERT peptide vaccination with adjuvant generated T-cells that did not recognize endogenously-processed telomerase (80).

Overall, these antigen-specific therapies have been well tolerated, with minimal toxicity, but only sporadic disease responses have been observed. The majority of these vaccines have been tested in the advanced disease setting. The optimal method of delivery of tumor antigens is not yet known, although many approaches have been tried (Table 1). These include adoptive immunotherapy with ex vivo expanded T-cells (81), peptide-based vaccines, proteins, RNA, DNA, and viral vectors such as vaccinia and fowlpox, that also

encode three costimulatory molecules [CD80/B7.1, ICAM-1, and LFA-3; designated TRICOM™ (59)].

Cellular-based vaccines

Vaccines based on whole autologous or allogeneic tumor cells have been combined with strong adjuvants or cytokines, since tumor cells themselves generally stimulate poor antigen presentation (82). Both autologous tumor cells (83–85) and allogeneic cell lines (86–88) have been used in clinical trials in breast cancer, with isolated clinical responses reported. Whole tumor cells have also been fused with dendritic cells (89). In murine models, GM-CSF was the most potent cytokine adjuvant for vaccination (90), and GM-CSF-secreting autologous and allogeneic vaccines are currently being evaluated in clinical trials in breast cancer.

TARGETING IMMUNE REGULATION

The focus of tumor immunology is shifting from targeting specific antigens to targeting the regulation of immune responses that result in impaired host immunity and tolerance to tumor antigens (Fig. 2 and Table 2). By activating co-stimulatory molecules and inhibiting molecules that down regulate immunity, effective T-cell immunity can be generated. By combining these approaches of targeted antigenic vaccination with “regulating the regulators,” it is hoped that specific anti-tumor T-cell-immunity can be generated.

Regulatory T cells

One mechanism of immune regulation is the activity of regulatory T-cells. These CD4+CD25+FoxP3+ T-cells inhibit other cellular immune responses and the development of autoimmunity (91, 92). Regulatory T-cells normally account for 5% to 10% of CD4+ lymphocytes in peripheral blood. In patients with breast cancer, however, regulatory T-cells are increased both in peripheral blood and in malignant effusions (93, 94). In a study of patients with ovarian cancer, elevated levels of regulatory T-cells in the tumor and in ascites were associated with poor survival (95).

Targeted therapeutics that specifically inhibit regulatory T-cells have been developed and are being tested in clinical trials. Denileukin diftitox (Ontak), which is a fusion of the full-length of interleukin-2 and the active portion of diphtheria toxin, can deplete regulatory T-cells by directly binding to CD25 (interleukin-2 receptor). In combination with RNA-transfected dendritic cells, Ontak augments T-cell responses in patients with renal-cell carcinoma (96) and in those with ovarian carcinoma (97).

Cytotoxic T-Lymphocyte-associated antigen-4 blockade

Cytotoxic T-lymphocyte-associated antigen (CTLA-4) is an inhibitory transmembrane molecule expressed on T-lymphocytes (Fig. 2). Activation of CTLA-4 strongly inhibits memory T-cell responses, as demonstrated by the development of lethal lymphoproliferative disease in CTLA-4 knockout mice (98, 99). The observation that CTLA-4 blockade can augment anti-tumor immunity in mouse models, has prompted an intense effort to develop antibody therapeutics that target CTLA-4.

Early-phase clinical trials have used anti-CTLA-4 mono-clonal antibodies in melanoma, and in ovarian, renal-cell, colon, and prostate cancers (101–106). CTLA-4 blockade has been associated with the development of significant Grade III/IV autoimmunity (dermatitis, colitis, hypophysitis) (106), but also with clinical responses in melanoma patients, including tumor necrosis (101–103). Notably, 9 of 29 patients with melanoma had either stable disease or extended periods without disease progression (23 to 36+ months) (102). Hodi et al. (101)

used autologous vaccination to prime T-cells and subsequent administration of an antibody that blocks CTLA-4 (MDX-010) to boost memory T-cell responses. This vaccination strategy resulted in significant tumor necrosis in three of seven patients with melanoma. Although these studies have focused primarily on malignant melanoma, CA-125 responses have been reported in patients with ovarian cancer (107) and PSA responses have been reported in hormone-refractory prostate cancer patients (105), arguing that augmentation of memory T-cell responses can be clinically effective in adenocarcinomas. It is not yet known whether the development of autoimmunity can be separated from the antitumor effects of this potent immunotherapeutic target.

CLINICAL ISSUES IN VACCINATION

There are several challenges that affect the development of breast cancer immunotherapies. Molecular typing of breast cancer (108) and genomic identification of breast cancer antigens (109) have made it clear that there are specific biologic types of breast cancer with different levels and patterns of tumor antigen expression. The identification of multiple antigenic targets in breast cancer (Table 1) has required the development of immunologic assays for careful monitoring of antigen-specific immune responses. There is no global assay for assessing immunocompetence, but antigen-specific T-cell responses can now be quantitatively measured with the use of flow cytometry using recombinant tetrameric HLA molecules (110), and plate-based ELISA and Elispot assays for T-celldependent cytokine secretion. Whole-cell based vaccines and modulators of immune regulation are more difficult to assess. Delayed-type hypersensitivity to vaccine can be tested in skin-biopsy specimens, and tumor-biopsy specimens can be examined for evidence of infiltrating lymphocytes, but identification of target antigens in complex vaccines and after targeted anti-immunoregulation remains difficult. Ideally, genome-wide and proteome-wide approaches to monitor immune responses will prove useful (27, 36).

Timing of vaccination

The timing of prior chemotherapy may be critical to the successful development of tumor-specific immunity. Cytotoxic chemotherapy has several effects on immune responses [reviewed in (111)]. It can abrogate existing immune responses, deplete regulatory T-cells, and induce a minimal residual disease state, thereby enhancing the potential effectiveness of immunotherapies. Specific chemotherapeutic agents, such as doxorubicin, paclitaxel, 5-fluorouracil, and cisplatin, have effects on the local tumor microenvironment, enhancing apoptosis, antigen presentation, or sensitivity to cytotoxic T-lymphocyte-mediated killing (112, 113). Cyclophosphamide, in particular, may decrease the function of CD4+CD25+ T-regulatory cells that inhibit immune responses (114). In addition, immunotherapy may enhance the efficacy of subsequent chemotherapies (115). These findings point to potential synergistic effects of chemotherapies and immunotherapies. Similarly, immunotherapies may be synergistic with other targeted therapeutics.

CONCLUSION

The development of cancer requires inhibition of effective immunity at multiple levels, from dysregulation of innate immune responses to active inhibition of adaptive immunity at the tumor microenvironment. As cancer progresses, so does the extent of immune dysregulation. Shifting the timing of vaccine delivery from the metastatic to the adjuvant setting (or earlier) should facilitate more effective anti-tumor immunity. To date, most vaccine strategies have focused on immune activation such as antigenic delivery, TLR activation by CpGs and adjuvant, and cytokine stimulation. However, the identification of immune regulatory pathways, such as B7-H1, B7-H4, CTLA-4, IDO, and regulatory T-cells has demonstrated that inhibition of immune regulation will be critical to establish effective anti-tumor

immunity. The successful development of breast cancer vaccines will require combinatorial therapies that target both breast-cancer specific immune activation and inhibition of immune tolerance.

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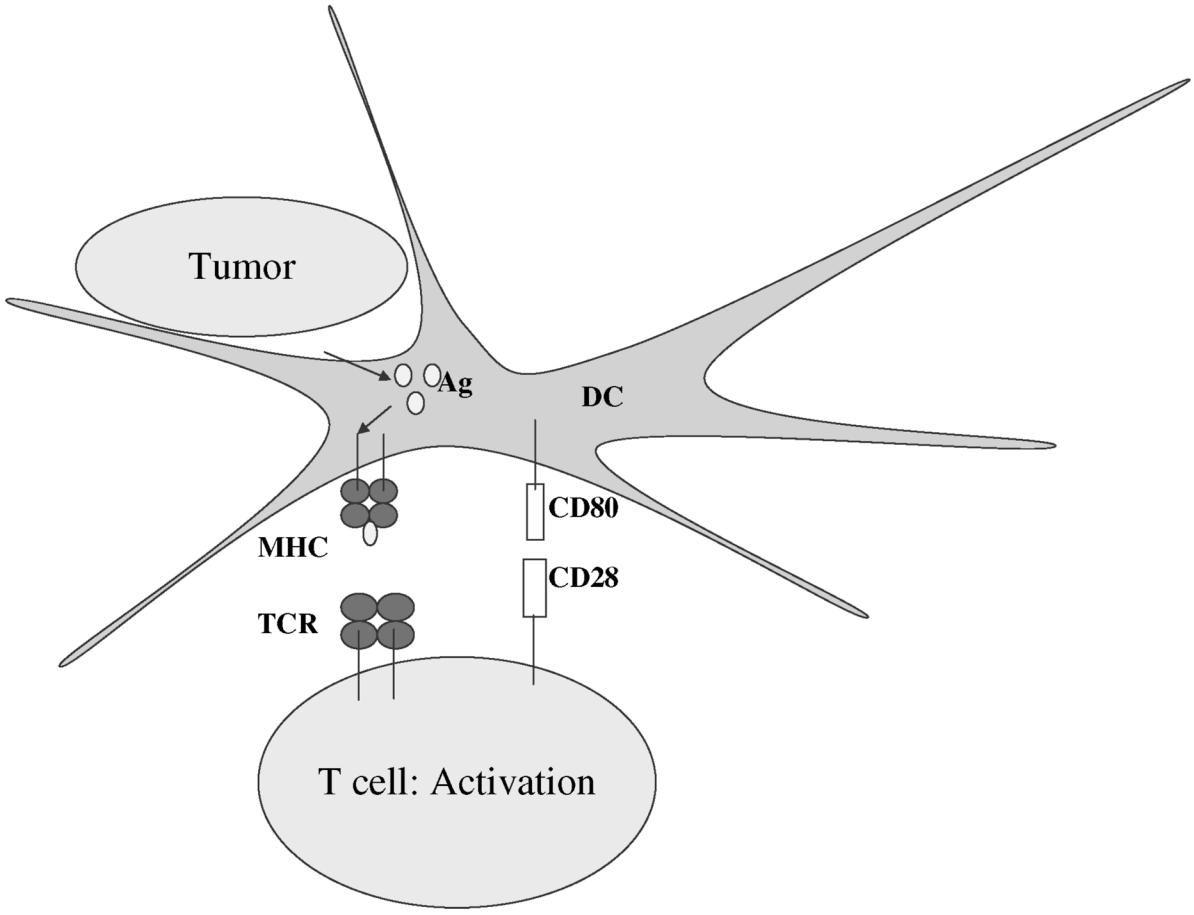

Figure 1.

Induction of T-cell immunity. Tumor antigens are endo-cytosed by immature dendritic cells, processed by proteases, and presented as peptides by MHC molecules to T cells. Expression of costimulatory molecules such as CD80 and CD86 are required for efficient priming of T cells via activation of CD28. *Abbreviations*: DC, dendritic cells; MHC, major histocompatibility complex; TCR, T-cell receptor.

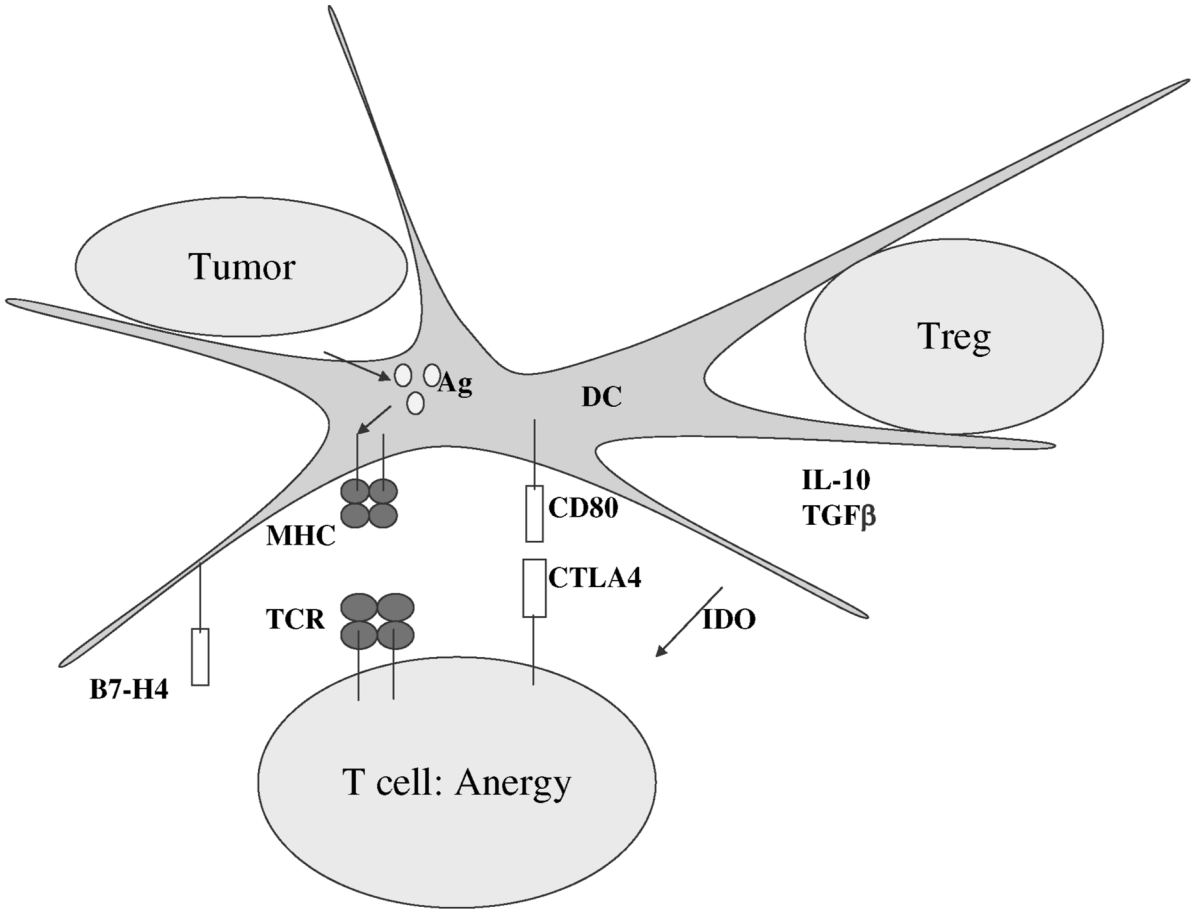

Figure 2.

Regulation of T-cell immunity. CD4+CD25+ regulatory T cells and cytokines in the tumor microenvironment such as IL-10 and TGF-β result in altered dendritic cell and effector T-cell function. This includes overexpression of inhibitory molecules, such as B7-H4 and indoleamine 2,3-dioxygenase, and activation of the T-cell inhibitory molecule, CTLA-4. These pathways result in T-cell anergy or tolerance. *Abbreviations*: CTLA4, cyto-toxic T-lymphocyte-associated antigen; DC, dendritic cell; IDO, in-doleamine 2,3-dioxygenase; MHC, major histocompatibility complex; TCR, T-cell receptor; TGF β, transforming growth factor.

Table 1

Breast Cancer Vaccination Strategies

Antigen	Antigen delivery	Adjuvant
HER2/neu	Peptide	GM-CSF
MUC-1	Protein	CpG
CEA	RNA	TRICOM™
Survivin	DNA	Dendritic cells
Telomerase	Vaccinia virus	QS-21
NY-ESO-1	Fowlpox virus	
Cyp1B1	Nanoparticles	
Cyclin B1	Expanded T cells	
Mammaglobin A	Cell fusion	
Carbohydrate antigens		
Autologous cells		
Allogeneic cells		

Table 2

Targets of Immune Regulation

Activating targets	Inhibitory targets
B7.1/CD80	Regulatory T cells
B7.2/CD86	FoxP3
CD40	CTLA-4
4-1BB	PD-L1/B7-H1
OX40	B7-H4
MICA, MICB	IDO
Toll-like receptors	TGF-β
TNF-alpha	IL-10
	FasL/CD95L

Abbreviations: TGF-β, transforming growth factor-β; TNF-alpha, tumor necrosis factor-alpha; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; IDO, indoleamine 2,3-dioxygenase.