Review

Breast Cancer Immunotherapy

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Breast cancer is a leading cause of cancer-related deaths in women worldwide. Although tumorectomy, radiotherapy, chemotherapy and hormone replacement therapy have been used for the treatment of breast cancer, there is no effective therapy for patients with invasive and metastatic breast cancer. Immunotherapy may be proved effective in treating patients with advanced breast cancer. Breast cancer immunotherapy includes antibody based immunotherapy, cancer vaccine immunotherapy, adoptive T cell transfer immunotherapy and T cell receptor gene transfer immunotherapy. Antibody based immunotherapy such as the monoclonal antibody against HER-2/neu (trastuzumab) is successfully used in the treatment of breast cancer patients with over-expressed HER-2/neu, however, HER-2/neu is over-expressed only in 25-30% of breast cancer patients. Cancer vaccine immunotherapy is a promising method to treat cancer patients. Cancer vaccines can be used to induce specific anti-tumor immunity in breast cancer patients, but cannot induce objective tumor regression. Adoptive T cell transfer immunotherapy is an effective method in the treatment of melanoma patients. Recent advances in anti-tumor T cell generation *ex vivo* and limited clinical trial data have made the feasibility of adoptive T cell transfer immunotherapy in the treatment of breast cancer patients. T cell receptor gene transfer can redirect the specificity of T cells. Chimeric receptor, scFv(anti-HER-2/neu)/zeta receptor, was successfully used to redirect cytotoxic T lymphocyte hybridoma cells to obtain anti-HER-2/neu positive tumor cells, suggesting the feasibility of treatment of breast cancer patients with T cell receptor gene transfer immunotherapy. Clinical trials will approve that immunotherapy is an effective method to cure breast cancer disease in the near future. *Cellular & Molecular Immunology*. 2004;1(4):247-255.

Key Words: breast cancer, antibody based immunotherapy, cancer vaccine, adoptive T cell transfer, T cell receptor, gene transfer immunotherapy

Introduction

Breast cancer is a leading cause of cancer-related death in women all over the world. It is estimated that about 1 in 8 women in the United States (approximately 12.8 percent) will develop breast cancer during their lifetime. Each year, more than 180,000 new cases of invasive breast cancer are diagnosed, with more than 40,000 deaths attributed to this disease in US. There are more than 1,000,000 new cases and 370,000 deaths yearly worldwide. Clearly, effective new treatment methods are needed to combat this disease. Although tumorectomy, radiotherapy, chemotherapy and hormone replacement therapy have been used for the treatment of breast cancer, there is no effective therapy for patients with invasive and metastatic breast cancer. Recent clinical trials in our branch at US National Cancer Institute demonstrate that adoptive T cell transfer immunotherapy is an effective approach to treat metastatic melanoma (1). Other clinical trials also confirm that adoptive T cell transfer immunotherapy is a promising method for the treatment of patients with metastatic melanoma (2). Animal models show breast tumor cells induce *in vivo* immune responses and thus lead to regression of breast cancer and systemic anti-tumor immunity (3, 4). Cell-mediated immune mechanisms have been detected in human breast cancers (5, 6). Therefore, immunotherapy may be effective in treating patients with breast cancer. This article reviews the current developments in human breast cancer immunotherapy including antibody based immunotherapy, cancer vaccine immunotherapy, adoptive T cell transfer immunotherapy and T cell receptor (TCR) gene transfer immunotherapy regarding their mechanisms, feasibility and application.

Antibody based immunotherapy

Mechanisms of antibody based immunotherapy are described in Figure 1. Antibodies specifically bind to the

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Abbreviations: CEA, carcinoembryonic antigen; CTL, cytotoxic T lymphocyte; HER-2/neu, human epidermal growth factor receptor 2; IL-2, interleukin-2; PBL, peripheral blood lymphocyte; TCR, T cell receptor; TIL, tumor infiltrating lymphocyte.

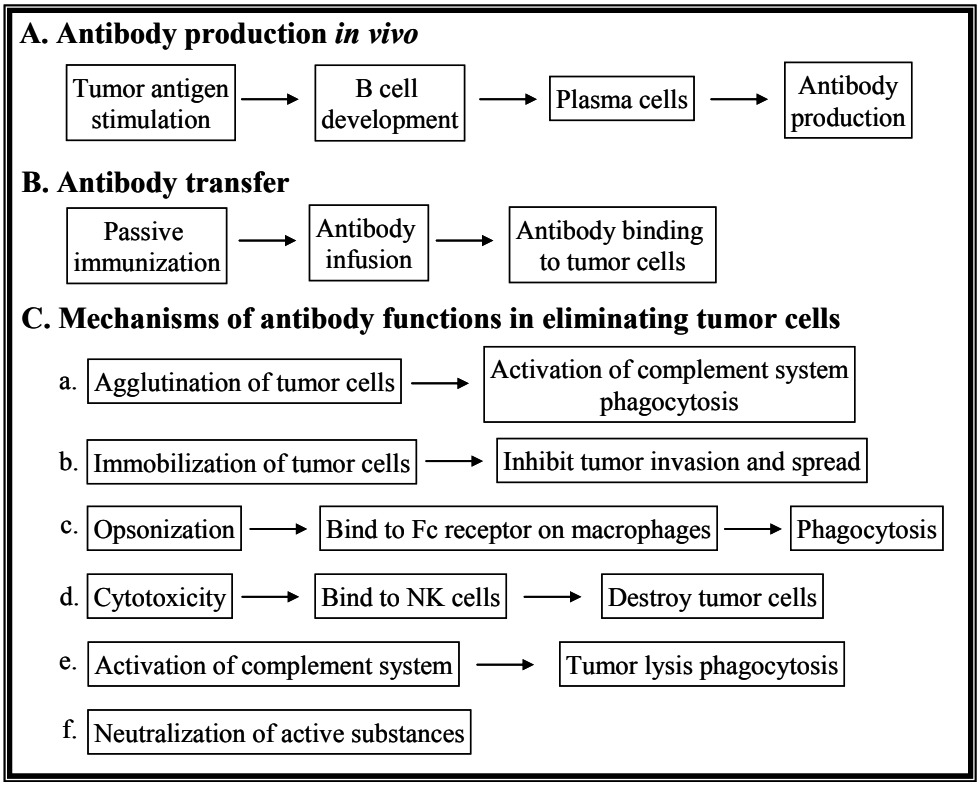

Figure 1. Mechanisms of antibody based immunotherapy. (A) Antibody production induced by tumor antigens in cancer patients. (B) Passive immunization by antibody transfer. (C) Mechanisms of antibody functions in eliminating tumor cells after antibodies bind to the antigens on tumor cells.

tumor antigens on the tumor cells. Multiple immune responses are elicited by the interaction between antibodies and antigens, leading to tumor cell eradication.

Two decades ago, several investigators reported that antibodies reactive with murine mammary tumor virus were present in sera of patients with breast cancer (7-9) and in sera of mammary carcinoma patients' healthy daughters (10). Circulating immune complexes containing antigens showing common epitopes with structural proteins of murine mammary tumor virus were detected in the blood sera of breast cancer patients (11). These data suggest that tumor antigen may induce humoral immune responses in breast cancer patients although the evidence for antibodies reactive with murine mammary tumor virus in sera of patients with breast cancer is controversial (12).

Recent data demonstrated that immunization with breast tumor antigens, HER-2/neu (human epidermal growth factor receptor 2) peptides and MUC-1 protein, successfully induced humoral immune response with anti-tumor activity in animal models (13-15). Moreover, 7% of the patients with advanced stage HER-2/neu over-expressing breast and ovarian cancers had detectable HER-2/neu specific IgG antibodies, range 1.2-8.9 μg/ml (16). High-titer HER-2/neu protein-specific antibody could be detected in 11% of the patients with early-stage breast cancer (17). In addition, spontaneous humoral immune responses against NY-ESO-1 are detected in a substantial proportion of patients with NY-ESO-1 positive cancers including breast cancer, prostate cancer, ovary cancer and melanoma (18-20). In a recent study, ninety-four distinct antigens reactive with serum IgG from breast cancer patients were identified by immunoscreening breast cancer-derived cDNA expression libraries (21), suggesting that multiple antibodies against breast tumor antigens are present in breast cancer patients. Therefore, humoral immune responses are truly induced in breast cancer patients. However, they are not related closely between antibody production and disease progression in patients with breast cancer. Probably, antibody titers may not be high enough to inhibit breast cancer development.

Monoclonal antibody technique makes antibody based immunotherapy feasible. The recombinant humanized anti-HER-2/neu monoclonal antibody, trastuzumab, was approved for clinical use in the United States in 1998. Clinical trials demonstrate that trastuzumab improves disease-free survival in patients with metastatic breast cancer (22-25). A clinical trial of 27 HER-2-over-expressing metastatic breast cancer patients treated with trastuzumab as a single agent showed that three patients had a complete and 3 a partial response, 3 had no change, 17 had progressive disease, one was not evaluated, and the overall response rate in the 26 patients with available data was 23.1% (25). Trastuzumab in combination with paclitaxel chemotherapy also resulted in a 25% improvement in overall survival compared with chemotherapy alone (23). A recent clinical trial showed that the overall response rate of trastuzumab in combination with vinorelbine chemotherapy reached up to 68% (26). Nevertheless, HER-2/neu is over-expressed only in 25-30% of breast cancer patients (27).

Another promising antibody may be bevacizumab, which is a monoclonal antibody against the vascular endothelial growth factor receptor (28). It has been found to have clinical benefit of 17% in phase II trials in breast cancer. A phase III randomized trial in breast cancer patients treated with the combination of bevacizumab and

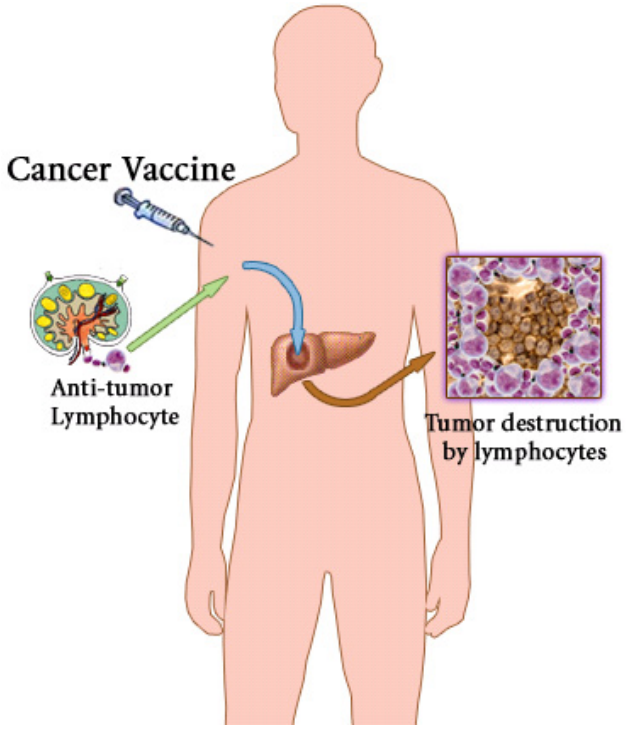

Figure 2. Mechanism of cancer vaccine immunotherapy. Cancer vaccines such as specific tumor antigen-derived peptides, proteins and DNAs are injected subcutaneously into cancer patients. It is believed that in lymph nodes, cancer vaccines are processed and presented by antigen presenting cells such as dendritic cells and macrophages to stimulate lymphocytes to develop into anti-tumor lymphocytes. Anti-tumor lymphocytes migrate to tumor sites and thus kill tumor cells.

capecitabine (an oral selectively tumor-activated fluoropyrimidine carbamate) showed that the overall response rate was significantly increased in the combination arm (30.2% *vs.* 19.1%), but progression free survival was not significantly different between the two groups (24).

MUC-1 is expressed on 90% of human breast cancers, which is an ideal target for antibody based immunotherapy. However, the preparation of monoclonal antibody against MUC-1 is still in development (29). No clinical trial data about anti-carcinoembryonic antigen (CEA) antibody based immunotherapy are available although CIBCHTB1, a monoclonal antibody against CEA, has been generated (30). The main reason is that CEA is a serum tumor marker, and serum CEA is elevated in 30-50% of patients with symptomatic metastatic breast cancer (31).

The information about the recent development of antibody based immunotherapy in other cancer diseases is also available (32). Although antibody based immunotherapy is a promising method in effective cancer treatment, its efficacy is not satisfied so far. Current efforts to improve antibody based immunotherapy focus on novel antibody development as well as antibody arming such as immunotoxins (antibody-toxin chimeric molecules) (33, 34), antibody radiolabeling (radioimmunotherapy) (35, 36) and drug-antibody conjugates (37, 38). Besides, monoclonal antibodies can be used in directed and efficient drug delivery and thus new antibody-directed enzyme prodrug therapy strategy (ADEPT) is developed (39, 40).

Cancer vaccine immunotherapy

Different from antibody based immunotherapy, T cell based immunotherapy uses anti-tumor cytotoxic T lymphocytes (CTLs) as weapons to kill tumor cells. T cell based immunotherapy includes cancer vaccine immunotherapy, adoptive T cell transfer immunotherapy and T cell receptor gene transfer immunotherapy.

Cancer patients really appreciate if cancer vaccine immunotherapy does work. So far, no any clinical trial data approve that cancer vaccine immunotherapy is an effective treatment for cancer diseases although many clinical trials of cancer vaccines are ongoing (41). However, cancer vaccine immunotherapy is still an ideal and desirable method to treat cancer diseases. Cancer vaccine immunotherapy uses specific tumor antigen-derived peptides, proteins, DNA, vectors, or peptide-, protein-, and tumor cell lysate-pulsed dendritic cells, or RNA- and DNA-transfected dendritic cells, or dendritic cell-tumor cell hybrids to immunize cancer patients to elicit antigen-specific anti-tumor cytotoxic T cells to lyse tumor cells expressing tumor antigens (Figure 2).

Tumor antigens are prerequisite for cancer vaccine immunotherapy. So far, over 1,000 different tumor antigens such as Mart-1, gp100 and NY-ESO-1 have been identified, mainly from melanoma (42-44). They can be classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens, and tumor-specific unique antigens. However, a limited number of tumor antigens from breast cancer have been identified. HER-2/neu (45), MUC-1 (46), NY-ESO-1 (47) are the examples of breast tumor antigens. More tumor antigens from breast cancer are demanded.

Overwhelming cancer vaccine immunotherapy clinical trials have been done or ongoing (48-50). The results demonstrate that cancer vaccines can induce vigorous specific both CD8 and CD4 T cell responses, however, no objective responses including tumor regression and prolonged disease-free survival have been observed. The most recent trial showed that 26 out of 29 patients (89%) with HER-2/neu-over-expressing breast or ovarian cancer received HER-2/neu protein vaccine developed HER-2/neu specific T-cell immunity, and the majority of patients (82%) also developed HER-2/neu-specific immunoglobulin G antibody immunity, but no clinical responses were described (51). Many other studies have confirmed this conclusion (52-54). NY-ESO-1, a cancer/testis antigen as a cancer vaccine, has been extensively used in clinical trial (41). Limited data indicate that primary NY-ESO-1 -specific $CD8^+$ T-cell responses can be induced by immunization with NY-ESO-1 peptides but the majority of NY-ESO-1-specific $CD8^+$ T cells exhibit lower functional avidity and no tumor reactivity (55, 56). Successful clinical trial in breast cancer patients using NY-ESO-1 vaccination has not been reported so far. MUC-1 is the most promising cancer vaccine to treat breast cancer patients. Repeated administration of TG1031 (an attenuated recombinant vaccinia virus containing sequences coding for human MUC-1 and the immune stimulatory cytokine IL-2) in patients with MUC-1-positive metastatic breast cancer

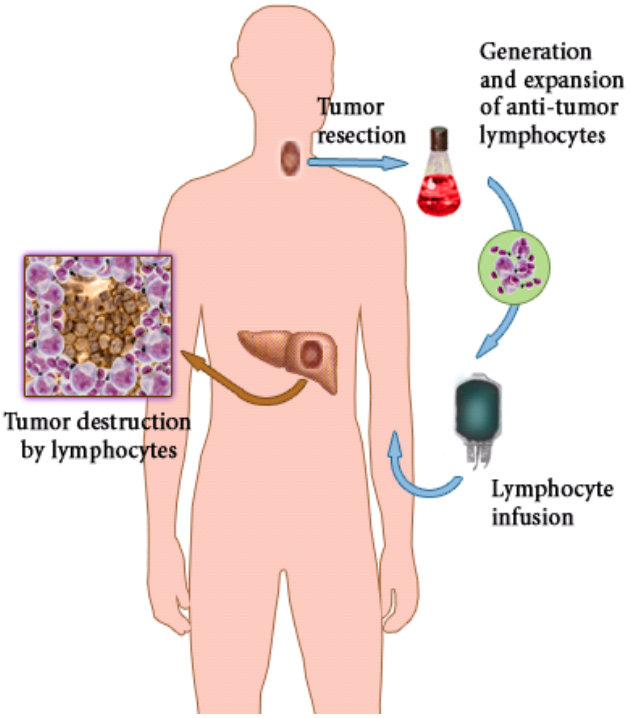

Figure 3. Mechanism of adoptive T cell transfer immunotherapy. Primary tumor tissues are surgically removed from cancer patients and used in generation of anti-tumor lymphocytes *in vitro*. Anti-tumor lymphocytes are then expanded to enough number of T cells. Usually 10^9-10^{11} T cells are transferred into cancer patients. Anti-tumor T cells migrate to tumor sites and thus kill tumor cells.

resulted in a partial tumor regression in 2 out of 28 patients, and partial regression lasted for 11 months in one patient and for 12 months in the second patient who then underwent surgical resection of her hepatic metastases (57). Immunization of MUC-1-positive patients with advanced or metastatic breast or lung cancer using dendritic cells loaded with MUC-1 antigens or tumor lysate elicited antigen-specific immunity and marked clinical effects such as reduction in tumor sizes or tumor marker levels or disappearance of malignant pleural effusion in 7 of the 9 MUC-1-positive cases (58). A synthetic STn (a carbohydrate associated with the MUC-1 mucin)-keyhole limpet hemocyanin (KLH) vaccine (Theratope(R)) induced a statistically significant survival difference between all breast cancer patients treated with the STn-KLH vaccine (overall median survival, 19.1 months; n = 50) and the retrospective control patients (overall median survival, 9.2 months; n = 104) (59). These studies demonstrate the potential use of MUC-1-based cancer vaccine immunotherapy in breast cancer although clinical efficacy remains limited.

Cancer vaccine immunotherapy is a promising method to treat cancer patients. Cancer vaccines can induce specific anti-tumor immunity, but cannot induce objective tumor regression. The main reason is that the frequency of tumor-specific T cells in cancer patients is too low to cause tumor regression. It is urgent to find out more potent cancer vaccines and improve immunization methods.

Adoptive T cell transfer immunotherapy

Adoptive immunotherapy was first introduced in the 1980s. Clinical trials in melanoma patients demonstrate that adoptive T cell transfer immunotherapy is a promising approach to inducing anti-tumor immune responses and cure cancer diseases (60). Adoptive T cell transfer immunotherapy involves in the generation of anti-tumor T lymphocytes from the primary tumor tissues of the patients with cancer diseases, T cell *ex vivo* expansion and activation, and subsequently autologous administration into cancer patients to cure cancer diseases (Figure 3).

Successful adoptive T cell transfer immunotherapy relies on the generation of anti-tumor T lymphocytes. T cells with specific anti-tumor reactivity can be generated from melanoma patients by *in vitro* culture of tumor infiltrating lymphocytes (TILs). It was reported that 860 attempted TIL cultures were generated from 90 sequential melanoma biopsy specimens from 62 human leukocyte antigen (HLA)-A2+ patients. Multiple independent TIL derived from a single tumor often exhibited substantial functional and phenotypic variation. Tumor specific activity was detected in TILs from 29 (81%) of 36 patients screened (61).

Recent clinical trails showed that the administration of anti-tumor T cells along with IL-2 after treatment of the melanoma patients with a nonmyeloablative lymphocyte depleting chemotherapy (60 mg/kg cyclophosphamide for 2 days and with 25 mg/m^2 fludarabine for 5 days prior to therapy) resulted in substantial regression of bulky metastatic melanomas at multiple sites including lung, liver, brain, and subcutaneous tissues. Six of 13 heavily pretreated patients, refractory to IL-2 treatment alone, achieved an objective cancer regression, and 4 additional

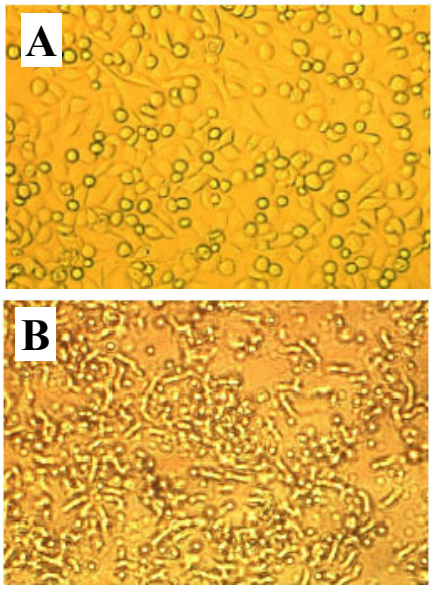

Figure 4. Generation of autologous tumor cells (A) and TILs (B) from the primary tumor tissue resected from a breast cancer patient.

patients had substantial mixed or minor responses (1). The results demonstrate that adoptive T cell transfer immunotherapy is an effective method to cure melanoma disease.

TILs can be generated from different cancer diseases. For example, lymphocytes were readily expanded from solid biopsies of non-small-cell lung cancer (NSCLC), and specific cytolytic and/or cytokine-releasing activity was observed in TILs from 3/15 solid tumor biopsies (20%) (62). Furthermore, several CTL clones from lymphocytes infiltrating a lung carcinoma of a patient with long survival were isolated, and functional studies indicated that these clones mediated a high HLA-A2.1-restricted cytotoxic activity against the autologous tumor cell line (63). Lymphocytes isolated from fresh human epithelial ovarian tumors can be expanded in the presence of recombinant IL-2, and some CD8 antigen-positive lymphocytes can lyse autologous fresh tumor cells (64). Severe infiltration of intra-tumor cell-infiltrating lymphocytes (ITCILs) was observed in 41.7% of high microsatellite instability sporadic colorectal cancer (MSI-H sCRC) patients, however, their anti-tumor specificity was unknown (65). γδT cells in tumor-infiltrating lymphocytes (γδTILs) were selectively expanded from patients with colorectal tumor and demonstrated marked cytotoxicities (66). A PTH-rP (a protein produced by prostate carcinoma and other epithelial cancers) specific CTL line was generated *in vitro* by cyclical stimulations with IL-2 and PTR-4 peptide-pulsed autologous dendritic cells, of HLA-A2.1(+) TILs derived from a patient with metastatic prostate carcinoma (67). Interestingly, the $CD3^+CD4^-CD8^+$ CTL line was established from TILs of a 3-mo-old child with Wilms' tumor, and this $CD3^+CD4^-CD8^+$ CTL line showed cytotoxicity against the HLA-A2402+ tumor cells including the autologous tumor, adenocarcinomas from various organs (colon, stomach, lung, and ovary), and an esophageal squamous cell carcinoma (68). Therefore, adoptive T cell transfer immunotherapy can be used to treat patients with melanoma as well as other cancer diseases.

It was reported that adoptive immunotherapy with tumor-associated lymphocytes (TALs) in combination with chemotherapy in patients with advanced-stage gastric cancer resulted in better overall survival of the treated patients (69). Another clinical trial reported that seven patients with advanced or recurrent epithelial ovarian cancers were treated using the adoptive transfer of anti-tumor TILs following a single *i.v.* injection of cyclophosphamide. One patient had a complete response and 4 patients had a partial response (14.3% and 57.1%, respectively). Regression of tumors in the ovary, liver, lung, and lymph node, both primaries and metastases, lasted for 3-5 months. In addition, 10 patients were treated alternately with a cisplatin-containing chemotherapeutic regimen and the adoptive transfer of TILs. Seven patients with a complete response and 2 patients with a partial response were observed. Four of the 7 patients with a complete response had no recurrence for longer than 15 months of follow-up (64). It appears that this experimental technique of adoptive transfer of TILs achieves high response rates even without recombinant IL-2 administration and that the prospect of combined therapy using TILs and cisplatin offers hope for increasing the cure rate and long-term survival of different cancer patients.

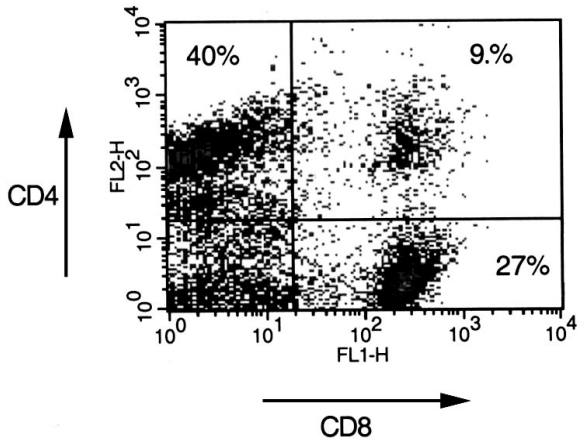

Figure 5. Composition of $CD4^+$ and $CD8^+$ T cells in a breast TIL.

Immunohistochemistry performed on frozen sections of 60 primary breast cancers by use of monoclonal antibodies to T lymphocytes (CD3), T-helper cells (CD4), cytotoxic T-cells (CD8), natural killer cells (CD56), IL-2 receptors (IL-2R), and major histocompatibility (MHC) class I antigen (HLA-ABC) and MHC class II antigen (HLA-DR) shows that all tested breast tumors are positive for CD3, CD4 and CD8, suggesting that TILs may be present in human breast cancer (5). TILs could be isolated from the enzyme digestion of primary human breast tumor specimen, however, they were consistently non-cytotoxic after isolation or culture in the presence of IL-2, and tumor lysate-pulsed mature DC could consistently restore tumor-specific lytic activity in non-cytotoxic breast cancer TILs (70). Breast TILs were generated in 15 of 19 cultures of primary breast cancers from the patients, became predominantly $CD4^+$ cells over time in culture, and were 73% $CD4^+$ and 21% $CD8^+$ (means) at 63 days (median). These TILs were poorly lytic in 4-hour ^{51}Cr release assays. Lysis of autologous tumor occurred in only one of 12 breast TILs. This lysis was low (15% at effector:target = 40:1) and was non-specific (non-major-histocompatibility-complex restricted). Cytokine secretion was tested by co-culturing TILs with autologous or allogeneic tumors for 24 hours (71). The results indicate that the generation of anti-tumor TILs from human breast cancer has not been successful.

Our research shows that anti-tumor TILs can be generated from primary tumor tissues of the patients with breast cancer. Figure 4 shows the growth of autologous tumor cells as well as TILs from the primary tumor tissue of a patient with breast cancer. This breast TIL is composed of both $CD4^+$ and $CD8^+$ T cells, and $CD4^+CD8^+$ double positive T cells are also present in this TIL (Figure 5), suggesting some naïve T cells exist in breast TILs. Furthermore, interferon-γ release assay demonstrates that breast TILs have tumor reactivity (Figure 6), indicating that anti-tumor breast TILs can be generated. Therefore, it is feasible to carry out adoptive T cell transfer immunotherapy in breast cancer patients.

Limited data show that adoptive T cell transfer immunotherapy is a promising method to cure breast

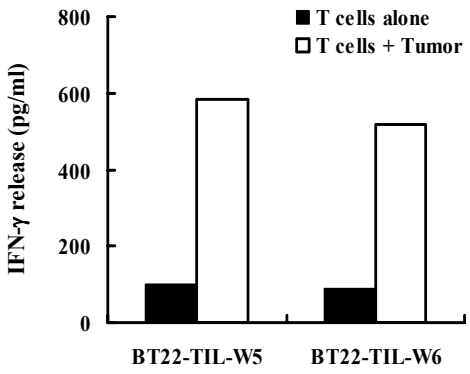

Figure 6. Tumor reactivity of two breast TIL micro-cultures, BT22-TIL-W5 and BT22-TIL-W6.

cancer disease. In a clinical trial, 81 breast cancer patients with malignant pleural effusions were treated by adoptive transfer of cultured autologous effusion lymphocytes, and 12 patients had survived 5 or more years (72). In another trial, 24 patients with malignant pleural effusions were treated with TIL and IL-2. CEA level decreased in all of the patients, and tumor cells disappeared in some patients (73). However, more clinical trials will still be needed to confirm this hypothesis.

T cell receptor gene transfer immunotherapy

As stated above, adoptive T cell transfer immunotherapy requires that T cells be isolated and expanded from individual patients. However, not all patients have accessible tumor lesions of sufficient size to provide an adequate number of T cells for expansion. In addition, tumor-specific TIL can only be obtained from 50% of TIL cultures. Consequently, adoptive T cell transfer immunotherapy is a viable treatment option for only 35% of melanoma patients, let alone a viable treatment for other cancer diseases. Alternative approaches would be definitely required to generate anti-tumor T cells instead of anti-tumor TILs. TCR gene transfer is a promising approach.

T cell receptor gene transfer immunotherapy is based on that the TCR is the only structure on the T-cell surface that defines its antigen-recognition potential although T cell function is regulated by means of numerous interactions with other cell types and soluble factors. Consequently, the transfer of TCRs into recipient cells can be used as a strategy for the passive transfer of T-cell immunity.

The first report about TCR gene transfer is that Jurkat T cells were transfected with the cDNAs encoding the full-length α and β TCR chains from the HLA-A2 restricted, melanoma-reactive T cell clone recognizing MART-1 antigen. Transfected Jurkat T cells recognized MART-1 peptides presented by T2 cells in a pattern and sensitivity equivalent to native MART-1-reactive T cells, but not HLA-A2+ melanoma cell lines (74). TCR with known anti-tumor reactivity could also be genetically introduced into primary human T lymphocytes. In this study, two distinct TCRs specific for the same HLA-A2-restricted peptide were derived from the melanocyte differentiation antigen, gp100, yet exhibiting different stringencies in peptide requirements. Retroviral transduction of primary human T lymphocytes with either one of the two sets of TCRαβ constructs enabled T lymphocytes to specifically kill native gp100+/HLA-A2+ tumor target cells as well as gp100 peptide-loaded HLA-A2+ tumor cells. Peptide titration studies revealed that the cytolytic efficiencies of the T lymphocyte transductants were in the same range as those of the parental CTL clones (75). The finding that two gp100-specific TCRs, derived from two different CTLs, can be functionally introduced into primary human T lymphocytes without loss of the antigen reactivity and fine peptide specificity, and holds great promise for the application of TCR gene transfer in cancer immunotherapy.

TCR gene transfer approach renders peripheral blood lymphocytes (PBLs) to acquire anti-tumor function. The genes encoding an αβTCR from a MART-1-specific, HLA-A2-restricted, human T cell clone were efficiently transferred and expressed in human PBL. These retrovirally transduced PBL cultures were MART-1 peptide reactive, and most cultures recognized HLA-A2+ melanoma lines (76). The α and β chains of the TCR from a highly avid anti-gp100 CTL clone were isolated and used to construct retroviral vectors to transduce PBL. The biological activity of transduced cells was confirmed by cytokine production following co-culture with stimulator cells pulsed with gp100 peptides and melanoma cell lines (77). Therefore, TCR gene transfer to patient PBL can produce CTL with anti-tumor reactivity *in vitro* and could potentially offer a treatment for patients with metastatic melanoma. This approach could also be applied to the treatment of other tumors and viral infections.

Transfer of T cell receptors directed against minor histocompatibility antigens (mHags), exclusively expressed on hematopoietic cells, could redirect virus-specific T cells toward antileukemic reactivity, without the loss of their original specificity. Generation of T cells with dual specificity may lead to survival of these TCR-transferred T cells for prolonged periods of time *in vivo* due to transactivation of the endogenous TCR of the tumor-reactive T cells by the latent presence of viral antigens. Furthermore, TCR transfer into restricted T cell populations, which are nonself reactive, will minimize the risk of autoimmunity (78). The dual specificity of these mHag-specific, TCR-redirected virus-specific T cells opens new possibilities for the treatment of hematological malignancies.

Chimeric receptors comprising of the TCRζ cytoplasmic signalling chain fused to an extracellular ligand-binding domain of a single-chain antibody (scFv), in which antibody determine the specificity, have also served as effective tools for redirecting CTLs against tumor cells. It was reported recently that chimeric T cell receptor permanently grafted TIL with predefined new specificity (79). In this report, a recombinant retroviral plasmid (pMSCVneo-Vhγ) was constructed by cloning VEGF121-hinger-FcRγ (Vhγ) into retroviral vector pMSCVneo. After packaging by PT67, the virus with high titer was used to infect TIL isolated from liver cancer tissues, and then MSCVneo-Vhγ-TIL was generated. TIL expressing Vhγ

could selectively recognize and kill vascular endothelial cells and tumor cells which express vascular endothelial growth factor receptor - KDR. In additions, a chimeric scFv/zeta gene composed of the variable regions of an HER-2/neu-specific monoclonal antibody (MAb) joined to the TCRζ chain was constructed. The scFv(anti-HER-2/neu)/zeta chimeric gene was successfully expressed as a functional surface receptor in the MD.45 CTL hybridoma (MD.45-HER/zeta). The scFv(anti-HER-2/neu)/zeta receptor was functionally active, since it triggered cytokine secretion by the MD.45-HER/zeta cells upon recognition of HER-2/neu-positive (+) tumor cell lines, or primary tumor cells from patients with HER-2/neu(+) cancers. The MD.45-HER/zeta-transduced cells also lysed HER-2/neu(+) target cells *in vitro* with high specificity (80). These data demonstrate the feasibility of treatment of breast cancer patients with HER-2/neu positive tumors using redirected CTL with the scFv (anti-HER-2/neu)/zeta chimeric receptor.

As stated above, TCR gene transfer immunotherapy holds great promise for cancer treatment, however, no any successful clinical trial data of TCR gene transfer immunotherapy are available so far. Some great progress has been made over recent years, but it will take years to make any significant clinical impact.

Summary

The goal of immunotherapy for cancer diseases remains the long-term eradication of tumor cells without adverse effects on normal tissue. Conventional approaches such as chemotherapy and radiotherapy are limited by both their lack of specificity and toxicity. Advances in understanding the nature of tumor-specific immune responses and mechanisms of tolerance induction have encouraged researchers and clinicians alike to develop a more refined approach to immune-mediated therapies. Successful attempts to produce monoclonal antibodies against unique breast cancer antigens for antibody based immunotherapy, to identify novel breast tumor antigens for breast cancer vaccine development, to generate anti-tumor TILs for adoptive T cell transfer immunotherapy and to redirect T cells with tumor specific TCR genes for TCR gene transfer immunotherapy have made the feasibility of breast cancer immunotherapy. Clinical trials will approve that immunotherapy is an effective method to cure breast cancer disease in the near future.

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