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Strategies to Improve Dendritic Cell-based Immunotherapy against Cancer

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Dendritic cells (DCs) play a pivotal role in T cell-mediated immunity and have been shown to induce strong antitumor immune responses *in vitro* and *in vivo*. Various approaches utilizing different vaccine cell formats, cell numbers, vaccination schedule, site of vaccination and maturation stages of DCs were investigated worldwide. While clinical trials have demonstrated the safety of such strategies, the clinical outcome was less than expected in most cases. This is due to in part host immunodeficiency imposed by tumors and immunoediting of tumor cells. To overcome these obstacles, new approaches to improve DC-mediated immunotherapeutic strategies are under investigation. First, functional enhancement of monocyte-derived DCs can be generated with using flt3-ligand (FL). Second, diverse antigenic determinants from heat shock-treated tumor cells may improve the immunogenicity of DC-based vaccines. Third, inclusion of *ex vivo* expanded NK/NKT cells in DC-based vaccines could be beneficial since the bidirectional interaction of these two cell types are known to enhance NK cell effector function and to induce DC maturation. Application of these approaches may induce a broadened antitumor immune response and thereby promote the elimination of tumor antigen-negative variant clones that had escaped immunosurveillance or undergone immunoediting. We are currently examining the feasibility of these immunotherapeutic approaches using a murine pancreatic cancer model system.

Key Words: Dendritic cells, tumor lysates, HSP, NK, immunotherapy, Flt-3 ligand

Despite tremendous efforts maid and significant progress achieved, the treatment of patients with malignancy remains an exceptionally difficult problem even. A number of mechanisms have been suggested for the escape of malignant cells from host immuno-surveillance.[1,2] Tumors may not express neo-antigens that are immunogenic or they may fail to express costimulatory molecules for the activation of T-cells. In addition certain tumors have been known to lack or be poor in expression of MHC antigens. Another reason for failure of immunosurveillance may be the fact that in the early development of a tumor, the amount of antigen may be too small to stimulate the immune system and due to the rapid proliferation of malignant cells, the immune system is quickly overwhelmed. In addition, tumors may evade the immune system by secreting immunosuppressive molecules[3,4] or induce immunotolerance against tumor-specific antigen through regulatory T cells.[5] Because of these characteristics, cancer can be viewed as an immunodeficiency disease.

Recently, with advances of understanding of the immune system, it has been clear that cell-mediated immune response, composed of DC, CTL and NK cells, plays a key role for tumor immunotherapy. Tumor vaccines aim at inducing CTL responses against tumor antigens to mount an immune response against tumors and the major concern for cancer immunotherapy is "how can we achieve an effective CTL response?" A number of studies have been revealed that the activation of effective CTL requires presentation of the antigen in the context of MHC molecules on the surface of antigen-presenting cells. DCs are the most effective antigen-presenting cells for the activation of naive T cells,[6,7] and tumor vaccinations with DCs have been shown to induce CTL responses and tumor regression in some patients.[8]

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With discovery of diverse tumor-associated antigens and development of the methods for generation of DC and CTLs, current attempts such as cancer vaccines or adoptive transfer of T cells have been tested in clinical field. Tumor vaccines using dendritic cells (DCs) have been shown to induce anti-tumor CTL responses. Since the first published clinical trial of dendritic cell vaccination in 1995, over a 100 independent studies describing more than 1000 vaccines have been published in peer-reviewed medical journals and reported in a number of symposia.[9,10] Trials have been performed in more than 15 countries and included patients with more than two dozen tumor types. Various approaches to formats of vaccines, vaccine cell numbers, vaccine schedule and site of vaccination were used. Until now, a variety of immunotherapeutic strategies have been applied with only modest clinical success. The insensitivity of majority of cancer patients to DC vaccines is likely due to the escape mechanisms operated by tumor cells[11-13] that are detrimental to optimal induction of an antitumor immune response. To overcome these obstacles, we have attempted three independent, but complentary, approaches.

Flt3 Ligand (FL) Augments the Functional Capacity of Monocyte-derived Dendritic Cells

Flt3 ligand, a hematopoietic growth factor, is a specific ligand for a receptor tyrosine kinase flt3 (flk-2, STK-1, CD135).[14] Major functions of FL on hematopoietic stem cells and progenitors are well-known[15] and its action on DC generation in mice[16] and human[17] are well established. Upon intravenous injection of FL to human monocytes as well as DCs are mobilized dramatically. While the receptor for FL is expressed on human monocytes,[18] its function on monocytes has not been studied thoroughly. Here, we determined the effects of FL on cell survival and growth of peripheral monocytes, and in particular, if FL could enhance the function of monocyte-derived dendritic cells (DCs) *in vitro*. We have found that FL promotes dose-dependent monocyte cell proliferation through MAPK pathway. The addition of FL to GM-CSF and IL-4 during DC generation not only increased the expression of surface molecules such as costimulatory molecule, CD86, but also improved the proliferation of the allogeneic T cells in a MLR. Our results suggest that monocytes represent circulating precursors of DCs and could have important implications for the design of future vaccine and immunotherapeutic strategies.

Whole Cell Lysates from Heat-treated Tumor Cells May Increase the Chance of Antigen Presentation

A critical issue in optimizing DC vaccines is the choice of tumor antigen for DC loading. Clinical vaccination trials for patients with malignant melanoma have demonstrated that vaccinating against a single antigen can induce tumor-specific CTLs but carries the risk of promoting tumor antigen escape variants.[19] A more recent trial showed that the generation of CTLs against three or more tumor antigens correlates with clinical response.[20] This approach, however, is limited by (1) peptide restriction to a given HLA type, (2) induction of CTL without TH1 response, (3) limitation of the immune response to the given antigen, and (4), no or only few available antigenic epitopes for many tumors. There are several sources of undefined tumor antigens for antitumor immunotherapy. Among them, the simplest approach to load DCs with antigens *in vitro* is the use of tumor cell lysates. The generation of CTL and TH clones with multiple specificities may be an advantage in heterogeneous tumors and could also reduce the risk of tumor escape variants. One of the potential drawbacks is that tumor cell lysates may contain high concentration of cytokines inhibitory to DC and can reduce the viability and function of DC thus potentially limiting the efficacy of DC-based vaccines. Furthermore, soluble antigens in the lysates have been known to be poorly presented on MHC class I molecule via cross-presentation. However, development of more efficient antigen-loading methods may be required to the effectiveness of tumor cell lysates as an antigen source.

Tumor-derived chaperone proteins (stress proteins or heat shock proteins; HSPs) have proven to be effective immunogens in animal models, and

their advantages as anti-cancer vaccines have been documented.[21] Chaperone proteins carry peptides as part of their escort duties and are important components of the antigen presentation machinery of a cell.[22] Recent reports indicate that the uptake of chaperones by APC is a specific, receptor-mediated process.[23,24] Because heat shock proteins have been shown to play a critical role in tumor rejection in mice, it is likely that the increased expression level of HSPs in tumor cell bodies will enhance their immunogenicity. On the basis of this theoretical background, lysate preparations from heat shock-treated tumor cells represent a promising alternative to tumor lysates in DC-based tumor vaccines. Heat-treatment of cancer cell lines induced high levels of inducible HSPs (Hsp70, Hsp90 and gp96) than control. Upon pulsing, these heat shock-treated tumor lysates increased the expression of CD86 and this effect was not due to endotoxin in the preparations. This approach may expand repertoire of specificity against tumor-derived peptides and may increase immunogeneicity of DC vaccine leading to stronger and broader CTL responses.

This approach was tested in a murine pancreatic cancer model using Panc02 cell line. Most cases of pancreatic cancer are inoperable when diagnosed and early diagnosis is extremely difficult due to the lack of specificity in clinical symptoms and signs. With the difficulty of early diagnosis, most cases of pancreatic cancer are presented initially as micro or grossly metastasized. Regarding the poor response rate of pancreatic cancer with surgery, chemotherapy or chemoradiotherapy, new treatment modalities, such as immunotherapy with DC or adoptive CTL transfer, are warranted for this type of cancer. Efforts in the immunotherapy of malignant disease concentrate on the induction and enhancement of immune responses against tumors. Compared with other tumors, such as renal cell carcinoma and malignant melanoma, pancreatic carcinoma is considered to be weakly immunogenic. In addition, pancreatic cancer cells exhibit several mechanisms of immune evasion. Nevertheless, as reported previously, CTL responses against pancreatic cancer cells can be induced *in vitro* using DCs.[25] Animal studies[26,27] and human cancer trials[19,28] have shown that specific T-cell responses against tumors as well as tumor regression can be achieved with vaccines based on DCs. Therefore, vaccination with DCs might offer a therapeutic option for patients with pancreatic carcinoma.

Vaccines of NK/NKT Cells with Dendritic Cells

For over last two decades, attempts have been made to cure cancer with adoptive cellular immunotherapy. A major obstacle to the successful application of this strategy has been the restricted availability of immunocompetent effector cells. Clinical trials of adoptive immunotherapy have focused mainly on lymphokine-activated killer cells (LAK), derived from peripheral blood mononuclear cells cultured with IL-2. While LAK cells combined with exogenous IL-2 *in vivo* have demonstrated anti-tumor efficacy in patients with certain types of tumors,[29] its application has been hampered by the inherently low antitumor activity *in vivo*[30] and by the difficulty of generating sufficient number of cells. LAK cells represent a heterogeneous population, in which the major effector cells are NK cells expressing CD56 and CD16. In contrast to CTL, NK and NKT cells, effector cells of the innate immune system, have the capacity to recognize and kill tumor cells in an antigen-independent manner. To this end, Bio-Cell of Korea established a protocol for expanding a population of activated human NK cells by use of chemically defined culture medium. Optimization of this cell expansion resulted in 100 - 200 fold expansion of total cell numbers in two weeks and of those 50 - 70% of cells were NK/NKT. As a prelude to clinical applications, these *ex vivo* expanded NK cells were tested for cytotoxicity capacity. There is evidence that DC can link innate and acquired effector mechanisms[31] either by direct cell-cell contacts or soluble factors.[32] Activation of effector cells of the innate immune system by DC might be a prerequisite for antitumor immunity in tumors lacking MHC class I molecules[33] and, under certain circumstances, for subsequent productive CTL responses. DC in conjunction with NK/NKT may be of clinical importance where innate as well as adaptive arms of immune responses can be induced. The ability to generate both NK effectors and mo-DCs in short-term

cultures may dramatically extend the therapeutic field of cancer immunotherapy.

While these DC-based vaccine approaches are still investigational and need to be addressed in additional experiments, continuous improvements of DC-based vaccines may lead to the successful translation of basic research into the clinical arena.

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