Cancer Immunotherapy

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A. Interaction between cancer cells and the immune system

It is well known that an intimate relationship between cancer cells and the immune system exists, and the understanding of their interaction is essential for the design of therapies that may enhance anti-tumor control by the immune system. A yin-yang phenomenon characterizes the relationship between cancer and the immune system: the immune system has a dual role in promoting as well as inhibiting cancer growth. Cells of both the innate immune system and adaptive immune system can be found infiltrating the microenvironment of cancer cells. While the innate system (neutrophils, macrophages) may enhance cancer growth and angiogenesis by the secretion of pro-inflammatory cytokines similar to the process of wound-healing (1), the adaptive immune system may also mount a specific immune response against tumor antigens, resulting in tumor destruction (2).

In general, macrophages that are found infiltrating cancer microenvironment are the immune-inhibitory type of cells that secrete immunosuppressive cytokines. These tumor-associated macrophages (TAMs) can also secrete proteases that promote cancer invasion as well as cytokines that promote tumor angiogenesis, and the presence of them in the tumor often signifies worse prognosis (3-4). Another type of cancer-promoting immune cell is the myeloid-derived suppressor cells (MDSC), which are immature myeloid cells that cannot differentiate into maturity in states of chronic inflammation. These MDSCs can inhibit anti-cancer immune response by enhancing the induction of regulatory T lymphocytes (Treg cells), which has immunosuppressive functions (5). These Foxp3 transcription factor expressing Treg cells mediate their functions under the stimulation by specific cytokines, such as IL-2 and TGF-b. They dampen anti-tumor immune response by secreting cytokines such as IL-10.

Anti-tumor immune response is an adaptive one, and antigen recognition is necessary in order to mount a destructive immune reaction against cancer. These tumor antigens can be viral antigen in virus-associated cancers, as well as altered self-proteins that become immunogenic as a result of increased expression. Evidence of adaptive anti-tumor immune response come from demonstration of tumor gene signature consistent with a T-cell signaling process (such as the upregulation of IFN-gamma), as well as the fact that the presence of infiltrating intratumoral

T-lymphocytes often signifies better prognosis (6-8).

There are other ways by which cancer cells evade destruction by the immune system. For example, immune response to peptide antigens derived from MAGE-6 protein in renal cell carcinoma was found to be switched to the Th2 pathway from the Th1 cytotoxicity, and this switch is tumor-specific because patients have Th1 response to other viral antigens (9). In fact, this Th2 skewing phenomenon has been demonstrated in many different types of cancers. Furthermore, Th2 lymphocytes may induce macrophages to differentiate into the tumor-promoting M2 phenotype in the tumor microenvironment. As mentioned above, tumor-infiltrating CD4/CD25/Foxp3 Treg cells play an important role in down-regulating anti-tumor immune response. The presence of Treg cells within the tumor represents worse prognosis in many different types of cancers, and reducing Treg cells by targeting CD25 was found to improve efficacy of cancer vaccines (10-11). To complicate matters more, in certain cancer types (such as head and neck and colon cancers), Treg cells may actually be a favorable prognostic factor. The exact mechanism by which this paradox occurs is not well understood, but it has been postulated that Treg cells in these situations may dampen the pro-proliferation inflammatory response induced by other immune cells. Interestingly, it has been suggested that T lymphocytes within the tumor microenvironment exhibit dynamic phenotypic features, and Th1 cells can behave like a Treg cell at different time points under different cytokine conditions. PD-1 (Programmed death-1) is a cell surface molecule expressed on T lymphocytes upon antigen recognition, and upon ligand binding (by PDL1 or PDL2 ligands), T cell response will be switched off. It has been shown that PD-1 ligands are present on certain tumor cells, which serve to switch off anti-tumor immune response and is also associated with worse prognosis (12), making PD-1 a potential target for the design of anti-cancer immunotherapy. CTLA-4 is another T-cell inhibitory molecule that has been employed as a molecular target as a form of immunotherapy. Clinical trial has shown that anti-CTLA4 monoclonal antibody can induce a response in melanoma especially in those patients who developed autoimmunity as a side effect (13). Immune evasion by cancer can also be mediated by the mesenchymal cells in the tumor microenvironment. It has been demonstrated that depletion of fibroblast-activating protein-alpha (FAP)-expressing cells in the tumor microenvironment resulted in a much enhanced anti-tumor immune response in an experimental model (14), suggesting that these FAP-expressing cells in the tumor stroma may suppress anti-cancer immune response. Exosomes have also been suggested to play a role in down-regulating anti-cancer immune response. These small membrane-bound particles may be shed from cancer cells and carry peptides

or RNA materials that convey a message to suppress anti-cancer immunity (15).

B. Adoptive T-cell Immunotherapy

Adoptive immunotherapy is a process that involves extraction of immune cells from patients' tumor, draining lymph node or peripheral blood, expansion of these immune cells in vivo specifically or non-specifically, and the re-infusion of these treated cells into patients in order to enhance anti-tumor immune effects. The concept that the immune system can have an anti-tumor effect came from observation that recombinase-deficient immunodeficient RAG2-/- mice were found to develop cancer more frequently in the early 1990s. The other observation was that a competent immune system can effectively suppress immunogenic, virus-associated tumors. It is now known that many self-antigens present on tumor cells can be recognized by T lymphocytes, especially in the setting of malignant melanoma. However, since T cells with self-specificity are generally deleted during the process of self-tolerance in the thymus, an anti-tumor response against tumor-associated self-antigens generally do not occur, except in situations that the characteristics of these self-antigens are altered, and some process to break this self-tolerance takes place. Tumor-specific cytotoxic T-cells have been detected in the blood of patients with melanoma without mounting any significant anti-tumor response. One strategy to enhance anti-tumor effect by the immune system is the in vivo sensitization and expansion of effector immune cells, including T cells, lymphokine-activated killer (LAK) cells and cytokine-induced killer lymphocytes (CIK).

LAK cells are produced by high-dose IL2 stimulation of peripheral blood mononuclear cells (which are presumed to contain common lymphocyte precursor cells), which then differentiate into CD56+ NK cell phenotype. Upon binding to MHC class I-related ligand that are overexpressed on tumor cells, killing of tumor cells may occur, although the usual inhibitory tolerance to self-class I MHC molecule can act as a counteracting effect. It has been postulated that some tumor cells may down-regulate MHC class I expression in order to escape antigen presentation to T-cells, and in so doing they get destroyed by NK cells due to the loss of MHC I inhibition. However, in clinical setting, such LAK strategy was not found to be very effective in controlling tumor growth (16). CIK cells are generated when peripheral blood mononuclear cells are stimulated by a combination of anti-CD3, IFN-gamma and IL2 to induce a population of CD3/CD56+ cells that have dual T cell and NK cell property, which can destroy tumor cells in a non-MHC restriction manner. Clinical

trial has shown encouraging results of CIK immunotherapy especially in the adjuvant setting (17).

Adoptive T-cell immunotherapy refers to the ex vivo expansion and stimulation of T cells from cancer patients with recombinant cytokines and specific TCR ligand antigens, and the reinfusion of treated cells into patients to induce an enhanced anti-tumor immune effect. Viral antigens are particularly desirable as a specific target for TCR in virus-related cancers, such as EBV-related malignancies. Malignant melanoma has been one of the most successful disease model for adoptive T cell immunotherapy in the past decade. Non-specific ex vivo expansion of T cells has not been very successful, and stimulation of T cell by specific tumor-associated antigen is needed for better therapeutic efficacy. It was also found that tumor-infiltrating lymphocytes may have more anti-tumor reactivity than peripheral blood polyclonal mononuclear cells. Such strategy has been employed in various clinical trials in melanoma and renal cell carcinoma with initial encouraging response, when tumor-infiltrating lymphocytes are expanded in vitro by IL2. However, the identification of tumor-associated antigen for more-specific T-cell therapy is still much needed. A variety of methods including SEREX, gene expression profiling have helped identified a list of tumor-associated antigens that have been shown to be immunogenic, and antibodies against these antigens can be detected in patients' blood. Tumor-associated antigens can be unaltered self-proteins, self-proteins that are overexpressed on tumor cells, antigens that are only expressed on tumor cells, mutated self-antigens as well as viral antigens. For successful anti-cancer immunotherapy, the dose of adoptively transferred cells is one of the most critical parameter, especially for diseases of significant volume, which is estimated to be up to 10^9 cells for human beings.

A number of clinical trials on adoptive T-cell immunotherapy in treating malignant melanoma have been reported with encouraging results in the past decade (18,19). Individual trials have own specific protocol. T cells could be harvested either from peripheral blood or from the tumor specimen, which are then subjected to ex-vivo expansion by IL-2, with or without specific selection against tumor antigens of MART-1 or gp100, and reinfusion in patients after lymphodepleting cytotoxic treatment. Clinical responses have been seen in patients with refractory diseases, though auto-immune complications were a significant concern. Further studies into how adoptive T-cell immunotherapy can be modified for enhanced effect are on-going, including strategies to prolong the survival of transferred cells and to enhance their specific anti-tumor effects, induction of stimulatory molecules or

cytokines as well as countering the effects of inhibitory co-stimulatory signals from both tumor cells and stromal cells.

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