REVIEW ARTICLE

Combined Immunotherapeutic and Chemotherapeutic Approaches to the Treatment of Cancer.

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Recent attempts to treat cancer with immunotherapy have offered some encouraging results. Although the use of adoptively transferred *ex vivo* activated lymphokine-activated killer (LAK) cells has met with only limited success, tumour-infiltrating lymphocytes (TIL) have shown considerable promise as a treatment for malignant melanoma. This new treatment modality, known as adoptive immunotherapy, is the result of advances in our understanding of the mechanisms of activation and proliferation of cytolytic effector cells such as natural killer (NK) cells and cytotoxic T-lymphocytes (CTL). Conventional treatment of cancer typically involves a combination of surgery, chemotherapy and radiotherapy. These modalities are quite effective at treating a number of different malignancies but each has its own limitations. Both malignant melanoma and adenocarcinoma are not very responsive to conventional therapeutic approaches, thus emphasizing the importance of developing new treatment options. Recently, clinical trials have demonstrated the efficacy of combining chemotherapy with immunotherapy in treating cancer. In addition, a number of *in vitro* and *in vivo* studies have shown that chemotherapeutic agents are able to sensitize tumour cells to lysis by activated lymphocytes. The mechanisms of drug-induced sensitivity of tumour cells are currently being explored. It appears that combined therapy regimens using anti-cancer drugs and adoptive immunotherapy may offer a more effective treatment option for cancer patients in the future.

INTRODUCTION

Conventional treatment of cancer has focused on surgical resection, chemotherapy and radiotherapy. However, some cancers, such as colorectal adenocarcinoma and metastatic melanoma, do not respond well to these standard approaches (1). Surgical procedures are useful in removing large tumour masses but are not effective in dealing with micrometastases (2). Chemotherapy and radiotherapy treatments are often given to eliminate micrometastases following surgical resection (3), but in many cases are ineffective and can be traumatic, leaving the patient in an immunocompromised condition (4).

Immunotherapy is a relatively new treatment option which involves harnessing the body's own immune system to destroy cancer cells. The theoretical advantages of this new approach to cancer treatment are quite substantial. A hallmark of many immune responses is specificity. Many of the toxicities related to chemotherapy are due to poor specificity because many anti-cancer drugs target rapidly proliferating cells such as stem cells, in addition to tumour cells (5).

Immunotherapeutic treatments that have been used in clinical trials include the use of activated lymphocytes termed lymphokine-activated killer (LAK) cells or tumour-infiltrating lymphocytes (TIL) (1,6). This involves removing lymphocytes from the patient, stimulating them *ex vivo* with cytokines

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and then reintroducing highly activated killer cells into the patient. Further clinical trials have explored the use of immunotherapy in conjunction with chemotherapy with encouraging results (7-9). The mechanisms by which immunotherapy synergizes with chemotherapy are currently being elucidated. This review focuses on the basic mechanisms of lymphocyte-mediated tumour cell cytolysis and recent attempts at combined immunotherapeutic and chemotherapeutic approaches to the treatment of cancer.

Lymphocyte activation and anti-tumour responses

The relationship between the immune system and the control of tumour metastasis is not clearly understood. It has been well documented that cytotoxic T-lymphocytes (CTL) can recognize and destroy autologous tumour cells in a major histocompatibility complex (MHC)-restricted manner (10-13). This provides evidence that T-cells mediate immune surveillance against malignant cells (14). However, other lymphoid cells such as natural killer (NK) cells can kill tumour targets in a non-MHC-restricted manner and do not require prior exposure to the target cell antigens to mediate killing (15). It has further been shown that NK cells have a greater cytolytic effect against tumour targets which have reduced expression of class I MHC molecules (16). Since many tumour cells exhibit reduced levels of surface class I MHC (17), the NK cell appears to have an important role in immune surveillance against cancer. NK cells stimulated with appropriate cytokines such as interleukin (IL)-2 can differentiate into LAK cells which have potent MHC-unrestricted cytotoxic effects on a variety of tumour cell lines (18). T-cells can be also be activated using IL-2 and anti-CD3 antibodies, which bind a portion of the T cell receptor mimicking antigen stimulation and leading to T-cell activation (19,20). These anti-CD3-activated T-cells are also MHC-unrestricted with respect to tumour killing activity and are able to lyse a wide variety of tumour cell lines (21).

Granule exocytosis from cytolytic effectors - the lethal hit

Following recognition and binding to a target cell, cytotoxic lymphocytes such as NK cells or CTL can mediate tumour cell killing through the exocytosis of cytotoxic granule contents. Although NK cells differ from cytotoxic T-cells in that they do not need antigen presented in association with class I MHC for activation, they do possess similar cytotoxic mechanisms to those of CTL (22). After a T-cell binds to a target cell, its granules are reoriented toward the region of the cell membrane contacting the target cell and the cytolytic granule contents are released into the region between the cells (23). The granules of CTL contain proteoglycans (24), serine proteases (granzymes) (25) and perforin (26). Perforin can form pores in the target cell membrane consisting of 12-18 monomeric perforin subunits (27). Disruption of the cell membrane in this manner has been demonstrated to induce cell death *in vitro* by osmotic means (28) but also facilitates the entry of granzymes into the target cell (29,30). Granzymes are a group of proteases that cause target cell death by triggering apoptosis, which is characterized by DNA fragmentation, chromatin condensation, extensive membrane blebbing and nuclear degradation (31,32).

ADOPTIVE IMMUNOTHERAPY

Many cancers such as metastatic melanoma are poorly responsive to conventional therapy, indicating a need for alternative treatments. Adoptive immunotherapy is a relatively new treatment modality for cancer that shows much promise. It involves removing lymphocytes from a patient, activating them *ex vivo* and reintroducing the activated killer cells back into the patient with the hope of reducing the tumour burden. It has been demonstrated that adoptive immunotherapy in murine models using LAK cells plus IL-2 can result in the regression of cancer (33-36). Clinical trials using IL-2 and LAK cells have also been effective in mediating cancer regression in patients with advanced colorectal carcinoma (37).

In vitro techniques have been developed to isolate and expand T-lymphocyte populations that have infiltrated into solid tumor masses (38). These TIL have been shown to be 50-100 times more effective than LAK cells in mediating tumour regression of metastatic melanoma in mice (39). TIL therapy has also been effective in treating human cancer. For example, regression of melanoma in 11 of 20 patients was observed in patients treated with TIL therapy (1).

Challenges for adoptive immunotherapy

Although adoptive immunotherapy offers promise in treating some cancers, it is not without technical problems. It is often a challenge to facilitate the proliferation of sufficient numbers of effector cells from a small population of tumour-reactive lymphocytes. In fact, it can take as long as 4-8 weeks to culture sufficient TIL for adoptive transfer (40). Toxicity is also a concern in that the high doses of IL-2 used to maintain tumouricidal activity of either LAK cells or TIL can result in toxity that can lead to chills, nausea and a generalized capillary permeability leak syndrome (41,42).

Although responses to immunotherapy have been seen in melanoma and renal cell carcinoma, complete tumour regression is rare (1,43,44). This indicates a definite need to combine immunotherapy with other more conventional cancer treatments to improve cure rates.

COMBINED IMMUNOTHERAPY AND CHEMOTHERAPY

Clinical trials involving combined immunotherapy and chemotherapy regimens have demonstrated improved cure rates for a variety of cancers (7,9,45). One mechanism by which chemotherapy regimens may synergize with immunotherapy is simply by reduction of tumour mass which facilitates lymphocyte infiltration into the tumour. In animal models, it has been noted that LAK cells accumulate four times more readily at the tumour site when administered in combination with cyclophosphamide or adriamycin (46). It has also been hypothesized that enhancement of immunotherapy following administration of chemotherapeutic drugs may be due to elimination of a suppressor cell population (47). However, *in vitro* studies have demonstrated that these are not the only mechanisms by which anti-cancer drugs enhance immunotherapy.

It has been shown *in vitro* that preliminary exposure to chemotherapeutic agents can enhance the killing of some tumour cells by lymphocytic effector cells (48-50). One chemotherapeutic drug which has shown considerable promise in combined chemo-immunotherapy regimens is cisplatin. A clinical trial comparing TIL therapy alone or in combination with cisplatin treatment of epithelial ovarian cancer showed complete response in 14% of patients with TIL therapy alone and 70% of patients treated with the combined therapy regimen (7). *In vitro* studies have also demonstrated that cisplatin can sensitize a variety of human tumour cells such as ovarian carcinoma, lung squamous carcinoma and gastric carcinoma cells to lymphocyte-mediated cytolysis (48-50). The molecular mechanisms by which cisplatin and other anti-cancer drugs facilitate lymphocyte-mediated killing of tumour cells are currently being investigated.

Mechanisms of drug-induced sensitization of tumour cells to immunotherapy

There are several mechanisms by which chemotherapeutic drugs can enhance cytolysis of tumour cells. Lymphocyte-mediated cytotoxicity ultimately leads to apoptosis which involves DNA fragmentation. Many chemotherapeutic drugs, such as cisplatin, have the capability to induce DNA lesions (51). Therefore, additive effects of chemoimmunotherapy may be due to cumulative DNA damage caused both by granzyme release by lymphocytes and chemotherapeutic drugs. However, more complex mechanisms of tumour cell sensitization which involve alterations in gene expression are becoming apparent.

Immunogenic surface proteins are thought to be induced by alkyl lysophospholipids, a group of anti-cancer compounds that target the cell membrane as their site of action. ET-18-OCH3 is a potent alkyl lysophospholipid which is non-cytotoxic at 25 μg/ml, but can sensitize K562 cells to killing by human LAK cells *in vitro* by inducing surface expression of heat shock protein, HSP72 (52). It addition, cisplatin can enhance LAK cell killing of Daudi and KATO-III cells by upregulating the expression of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function antigen-3 (LFA-3), both of which are important in LAK cell adhesion to tumour targets (53). Therefore, it is clear that drug-induced sensitization of tumour cells involves a number of alterations in expression of cell-surface proteins which facilitates lymphocyte-mediated killing.

CONCLUSION

It is apparent that immunotherapy for cancer is rapidly approaching the status of more conventional treatment protocols, such as surgery or radiotherapy. Continued research into the mechanisms of lymphocyte-mediated anti-tumour responses will ultimately lead to more potent effector cells and improved cure rates. Combining immunotherapy with chemotherapy has already yielded promising results in clinical trials and the mechanisms of drug-induced tumour sensitivity to immune-mediated attack are rapidly being elucidated. As these mechanisms become clear they will provide the basis for new treatments of cancers that are currently intractable.

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