
Studies on Dendritic Cell derived TNF
superfamily ligands in Leukemia & Lymphoma:
Role of Cytokines

Ph.D. Thesis Abstract

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Introduction

A growing list of defined tumor-antigens opens the way to antigen specific immunotherapy of cancer. However, current approaches are often limited in their potential to induce an effective anti-tumor response. Cancer immunotherapy attempts to harness the power and specificity of the immune system to treat tumors. The molecular identification of human cancer-specific antigens has allowed the development of antigen-specific immunotherapy. In one approach, autologous antigen-specific T cells were expanded ex vivo and then re-infused into the patients. Another approach is through vaccination; that is, the provision of an antigen together with an adjuvant to elicit therapeutic T cell response in vivo. Dendritic cells (DC) are natural adjuvants for the induction of antigen specific T cell response. Owing to their properties, DC are often called 'nature's adjuvants' and thus have become the natural agents for antigen delivery. After four decades of research, it is now clear that DC are at the centre of the immune system owing to their ability to control both immune tolerance and immunity. Thus, DC are an essential target in efforts to generate therapeutic immunity against cancer.

This thesis is based on the several reports of DC based immunotherapy against various cancers in clinical and experimental tumor model. Understanding the molecular mechanisms involved in tumorigenesis and the influence of cellular mechanisms including the role of cytokines on clinical outcome may provide specific molecular markers for targeted therapy. DC has been regarded as the professional antigen presenting cell although its direct cytotoxic role has recently been under intense research by several investigators. Elucidation of the complex network of signaling pathways that develop in DC induced immunotherapy and associated cytokine network could provide a greater insight in the molecular mechanism of the cancer.

In view of these, an attempt was made to study the direct role of cytokine activated DC with respect to growth inhibition, cytotoxicity and cell death

mechanism(s) against normal as well as drug (Doxorubicin) resistance Dalton's lymphoma (a highly aggressive murine lymphoma) both in vitro and *in vivo*. The critical effector function of DC was also extensively studied in a panel of parental and doxorubicin resistant human leukemia cell lines (K-562, JE-6.1, U937, THP-1) and CML patient derived primary CML cells in order to validate the experimental results. DC was also used in adoptive cell therapy for immunotherapeutic purpose against experimental murine lymphoma called Dalton's lymphoma (DL), generated in susceptible mouse. Following specific objectives were formulated for the present study:

- To study the antitumor potential of immature and IL-15 activated DC with respect to growth inhibition and cytotoxicity against murine lymphoma & human leukemia in vitro.
- Identification of mechanisms of antitumor potential of DC whether mediated via cytokine or by apoptosis.
- To define the TNF superfamily members like TRAIL and TNF- α in DC induced signaling events in lymphoma and leukemia.
- To study the IL-15 stimulated activated DC in immunotherapy against the lymphoma in experimental murine model.

The observations and results reported in the thesis is divided into various chapters, and demonstrated the tumoricidal properties of DC derived TNF-related apoptosis-inducing ligand (TRAIL) and Tumor necrosis factor- α (TNF- α) following stimulation with IL-15 against a highly invasive and vigorously metastatic CD3-CD11b⁺ CD19⁺ murine lymphoma called Dalton Lymphoma (DL).

Chapter 1: *Dendritic cell derived TRAIL is critical for anti-tumor activity against lymphoma & leukemia*

Effector functions in tumor resistance by dendritic cell (DC) are less well characterized. In this chapter, we describe that the murine DC upon stimulation with recombinant IL-15 in vitro or in vivo, expresses TNF

superfamily member TRAIL which mediates cytotoxicity and growth inhibition against Dalton's lymphoma (DL) via apoptosis. Presence of tumor lysate or intact tumor cells significantly reduces the DC mediated tumoricidal effect, possibly via masking and down-regulating TRAIL in DC. The antitumor effect of DC derived TRAIL was further augmented by deactivation of STAT3 in tumor cells by Cucurbitacin I (a specific STAT3 inhibitor), which makes DL cells more susceptible to DC derived TRAIL. Treatment of tumor cells with Cucurbitacin I upregulates TRAIL receptor expression in addition to activation of caspases. Compared to naïve DC, DC from tumor bearing mice are significantly impaired in TRAIL expression and consequent antitumor functions against DL which was partially restored by activation with IL-15 or LPS. Priming with recombinant IL-15 prolong the survival of tumor bearing mice treated with Cucurbitacin I. Naïve peripheral blood DC derived from chronic myeloid leukemia (CML) patients have significant impairment in expression of TRAIL and consequent tumoricidal properties against TRAIL sensitive lymphoma cell lines and primary tumor cells compared to normal control.

Chapter 2: *Inhibition of JAK/STAT3 signaling augments dendritic cell mediated anti-tumor activity: Role of TNF- α*

The current chapter was motivated by the need for effective therapeutic protocol against aggressive and highly metastatic lymphoma which often causes death and offers no chance of survival for the patients. This was further complicated by the emergence of drug resistance cases which makes treatment opportunity circumscribed. We could speculate that with the emergence of drug resistance, DC derived TNF- α subjected to downregulation with consequent restrain in immune responses. The current work will be helpful in designing therapeutic strategies against the drug resistant lymphoma based on cell therapy or boosting immune system via a combination of chemotherapy and cytokine therapy. Besides that, the current work also correlates the network of cell signaling events where TNF- α integrate multiple cellular events as a mediator.

Tumor necrosis factor- α (TNF- α) exhibits extensive antitumor activity, changes endothelial barrier function, decreases tumor interstitial pressure, and mediates immune responses. Although disputed by some, increasing evidences suggests that TNF- α synergizes with traditional chemotherapeutic drugs to exert a heightened antitumor effect. The present study investigated the antitumor efficacy of recombinant IL-15 (rIL-15) in combination with the STAT3 inhibitor Cucurbitacin-I in a doxorubicin (DOX)-resistant murine lymphoma model. TNF- α is downregulated in dendritic cells (DC) from mice with Dalton's lymphoma (DL) and shows an inverse relationship with disease progression. Doxorubicin-resistant (DOX-R) DL cells have elevated levels of Bcl-2 and Mcl-1 and increased phosphorylation of STAT3. These cells are refractory to DC-derived TNF- α . DOX-R DL is susceptible to DC-derived TNF- α upon stimulation with the STAT3 inhibitor Cucurbitacin-I, which downregulates STAT3 and other survival molecules. The combined treatment of low dose of Cucurbitacin-I and rIL-15 is ineffective in mice with DOX-R DL, but a similar therapy prolongs the survival of mice transplanted with parental DL. DOX-R DL responds to therapy with high doses of Cucurbitacin-I and rIL-15. DC derived from surviving mice following therapy regained their tumoricidal properties with respect to growth inhibition and killing of DL tumor cells. Similar to DL, DC derived from CML patients are impaired in TNF- α expression and are unable to restrict the growth of drug-resistant lymphoma and leukemia cells. This combination approach could be used as a new therapeutic strategy for aggressive and highly metastatic DOX-R lymphoma.

Chapter 3: *Establishment of protocol for dendritic cell based immunotherapy against lymphoma*

Adoptive cell therapy using dendritic cell (DC) is a strategy to deliver tumor antigens in cancer immunotherapy. Co-delivery of antigens to DC with essential components like genes encoding cytokines, chemokines, and other molecules or stimulation with recombinant cytokines is a potential method for designing an effective tumor vaccine protocol. Here, we describe the stimulation of bone marrow derived DC with recombinant interleukin-15 (IL-

15) in presence of intact soluble antigen (whole cell lysate) from tumor cells in an experimental animal model.

Chapter 4: *Adoptive transfer of whole tumor lysate pulsed IL-15 activated dendritic cell combined with Cucurbitacin I and rIL-15 cures highly aggressive murine lymphoma*

AKR/J Mice with Dalton's lymphoma (DL) was treated with recombinant IL-15 (rIL-15) activated autologous dendritic cell (DC), pulsed with whole tumor cell lysate in presence or absence of suboptimal dose of specific STAT3 inhibitor Cucurbitacin I. One group of treated mice was also received rIL-15 in order to boost the DC based adoptive cell therapy (ACT). Kaplan-Mayer survival analysis, multiple immunological and enzymological parameters were assessed in order to demonstrate the efficacy of vaccination protocol. Therapy with tumor lysate pulsed rIL-15 activated DC plus Cucurbitacin I prolong the survival of the tumor bearing mice significantly but fails to provide complete cure from the disease. Additional treatment of vaccinated mice with rIL-15 dramatically improves the therapeutic efficacy, and cures >75% of vaccinated group with no relapse for rest of their life. DC derived from the successfully vaccinated mice regained the anti-tumor potential against DL with respect to growth inhibition and cytotoxicity. Both CD4⁺ and CD8⁺ T cells were mobilized in metastatic organs of surviving vaccinated mice in large numbers, and demonstrate antigen specific proliferation and cytotoxicity against tumor cells. ACT also augments the DC function by up-regulating TRAIL and TNF- α expression. Besides that the combinatorial immunotherapy restores the levels of antioxidant enzymes and serum liver function enzyme activities, which were severely repressed in tumor bearing mice with no treatment. Effective vaccination for complete cure against aggressive lymphoma requires DC based ACT in combination with chemotherapy and cytokine therapy.

Conclusion

In conclusion, we postulated that in order to generate a comprehensive anti-tumor immunity, dendritic cells require optimum activation via cytokine signaling which enhances the expression of major tumoricidal factor like

TRAIL, TNF- α and simultaneous application of a second strategy in the form of chemotherapy could limit the proliferation of tumor cells. Simultaneous priming of dendritic cells by IL-15 and treatment with specific inhibitor against STA3, we showed that a successful therapeutic outcome is possible in early stages of cancer with minimum tumor mass and antigenic load. We propose a novel treatment strategy which may be beneficial against tumor progression and metastasis.