行政院國家科學委員會專題研究計畫　期中進度報告

以樹突細胞為主的免疫療法治療在小鼠的殘餘血癌疾病模式(1/2)

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中文摘要

我們的初步資料顯示，腫瘤抗原-pulsed 的樹狀細胞能夠引起專一性及保護性的抗血癌效果，並且能夠明顯的延長有植入小鼠 RL male 1 血癌細胞株的 BALB/c 小鼠的存活時間。根據以上研究資料，我們提出此研究計劃來測試以樹狀細胞為主的免疫療法，用來作為臨床化學療法後的殘餘腫瘤的輔助治療，以期避免腫瘤的再復發。

在今年，我們已建立小鼠血癌疾病模式（圖一），並藉由化學治療來治療已建立起明顯的血癌疾病，使其達到緩解（圖二），並觀察其會復發與否（圖三），以研究由骨髓細胞所衍生的樹狀細胞所引發的腫瘤專一性免疫反應。我們將藉由檢定各種體外的免疫反應和體內的抗殘餘血癌效果，以評估各種的 pulsed(TA 或 TA/adeno-IL12)及非 pulsed 的樹狀細胞的效用，及最佳的疫苗施打方案。

Abstract

Our preliminary results have demonstrated that vaccination with tumor antigen(TA)-pulsed DCs could induce a specific protective anti-leukemia effect and significantly prolonged survival of BALB/c mice that were challenged with murine RL male 1 leukemia. Based on our preliminary data, we propose this study to test that the capacity of DC-based immunization may have clinical implications as the adjuvant treatment of residual leukemia after the traditional induction chemotherapy to prevent the recurrence of the disease.

In this year, we have established the murine leukemia model (Figure 1) and by initial chemotherapy for the established frank leukemia status to induce remission (Figure 2), monitor relapse or not(Figure 3) and investigate the effect of BM-derived DCs in inducing tumor-specific immune response. We will evaluate the efficiency of various pulsed (TA or TA/adeno-IL12) or unpulsed-DCs by both *in vitro* immune response assays and *in vivo* anti-residual leukemia effect. We will also elucidate the novel optimal immunization schedule for inducing the competent anti-residual tumor immune response.

Brief Background, Rationale

The acute lymphoblastic leukemia (ALL) is the most popular malignancy among the childhood cancers worldwide. The cytotoxic chemotherapy drugs has increased the complete remission rate (into the residual leukemia status) to greater than 95%, however, the event of relapse after the achievement of remission is the major cause for the much lower long-term event-free survival rate (about 75% for standard risk group, 58.7% for high risk group and 50.4% for the very high risk group). Improved results may require the incorporation of additional therapies. The immune-mediated antileukemic reaction may qualify in this regard.

Dendritic cells (DCs), which phagocytose antigens and subsequently proliferate and migrate, may be the most powerful antigen-presenting cells that activate naive T cells through the MHC and costimulatory molecules [9]. Many experimental results have encouraged the clinical attempts to exploit DCs for cellular immunotherapy against human cancers. Bendandi M et al have demonstrated the complete molecular remissions induced by patient-specific DC immunotherapy plus GM-CSF for the first clinical complete remission lymphoma patients after standard chemotherapy [19]. Based on the above rationae, ALL immunotherapy during the phase of minimal residual disease (MRD) seems to be a possible and promising approach.

Results and Discussion

We have used different numbers of RL male 1 murine leukemia cells to inoculate BALB/c mice subcutaneously (table 1). This inoculation model worked well. The duration between the inoculation date and the day to begin treatment with chemotherapy agents are shown in table 1. The representative subcutaneous tumor mass is shown in Figure 1.

After chemotherapy (table 1), all mice's tumor masses disappeared grossly(Figure 2). However, some relapsed subcutaneously in different site other than the original one(Figure 3). We also checked the potential dissemination of the tumor cells within various organs microscopically. There were tumor cells found in lympho- node, liver, spleen, and interestingly kidney as shown in the following pictures.

Currently, we are trying to find the optimal chemotherapy agents, dosage and schedule to obtain the good remission rate and also reasonable relapse rate for the further DC immunotherapy.

Table 1

Mice no.	6	25	6
Inoculated cancer cell no.	5×10^6	6×10^6	6×10^6
Inoculation period (day) (mean±SD)	15±1.41	14	16
Tumor size (cm) (mean± SD)	1.22±0.53	1.42±0.49	1.43±0.29
Chemotherapy agents & dosage	Ara-c:600 μ g/day Rinderon:80 μ g/day MTX:125 μ g/week	Ara-c:600 μ g/day Rinderon:80 μ g/day MTX:125 μ g/week	Ara-c:100 μ g/day
Treatment period (day)	6.75	5	7
Outcome remission (duration)	60-~	~	~
Relapse (隻)	1	1	0
Relapse (day)	48	34	

Self-Estimation

We have completed most of the expected goals for the first year. However, it took time to monitor the relapse of tumors after various chemotherapy agents, dosage and schedules.

Reference

1. Fujii S. Hamada H. Fujimoto K. Shimomura T. Kawakita M. Activated dendritic cells from bone marrow cells of mice receiving cytokine-expressing tumor cells are associated with the enhanced survival of mice bearing syngeneic tumors. *Blood. 93(12):4328-35, 1999 Jun 15.*

2. Bendandi M. Gocke CD. Kobrin CB. Benko FA. Sternas LA. Pennington R. Watson TM. Reynolds CW. Gause BL. Duffey PL. Jaffe ES. Creekmore SP. Longo DL. Kwak LW. Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma.[comment]. *Nature Medicine. 5(10):1171-7, 1999 Oct.*

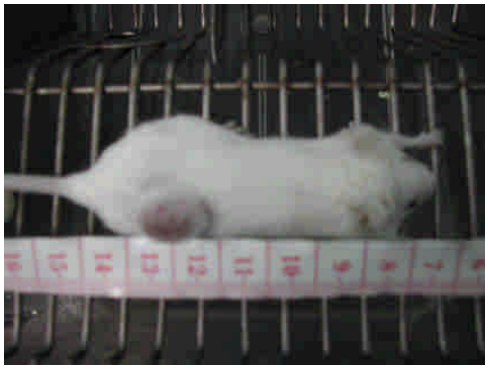
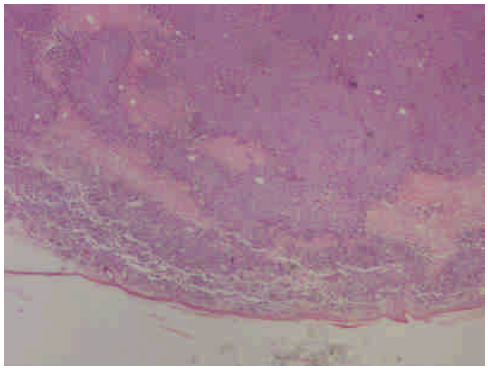


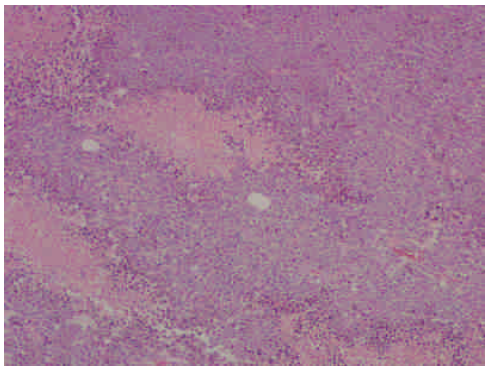
Figure 1
Tumor mass before chemotherapy treatment.

Figure 2
Tumor disappeared after chemotherapy treatment.

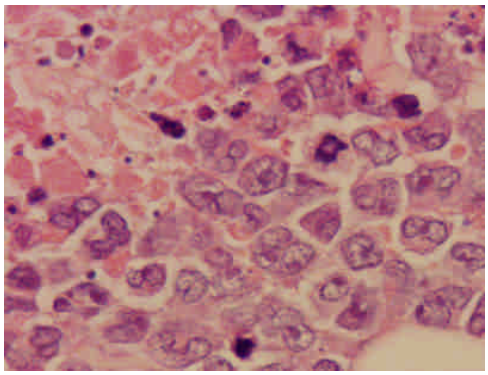
Figure 3
Tumor relapsed in different site.



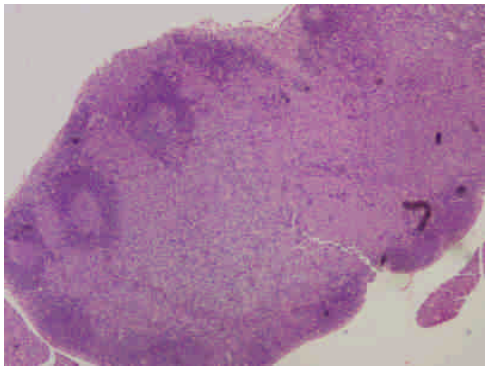
4x10
Relapsed tumor mass with hemorrhage.



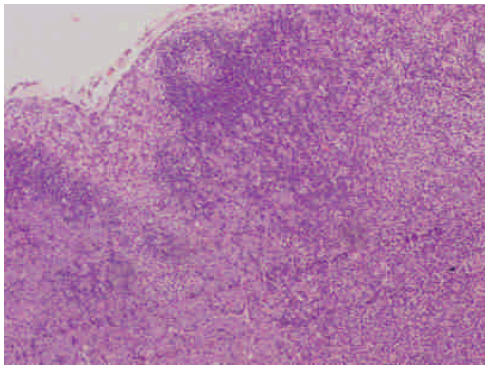
10x10
Relapsed tumor mass with hemorrhage.



100x10
Relapsed tumor mass with hemorrhage.



4x10
Relapsed tumor cell infiltrates in lympho-node.



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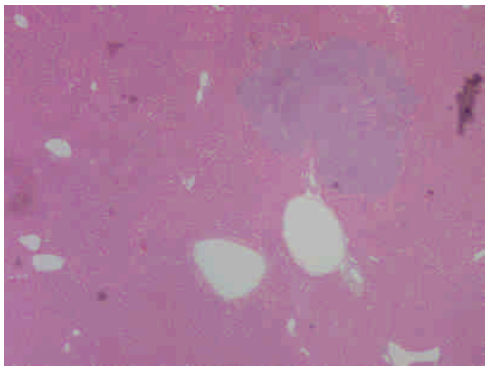
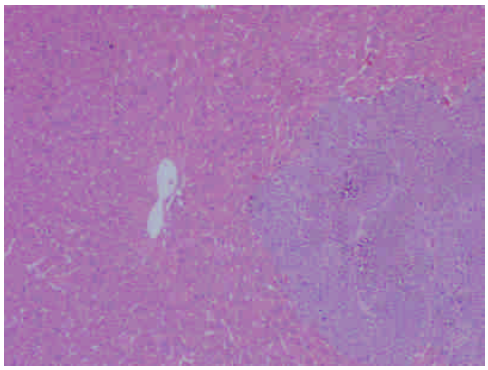
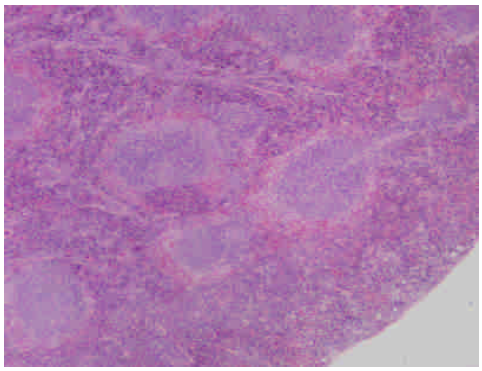
Relapsed tumor cell infiltrates in lympho-node.



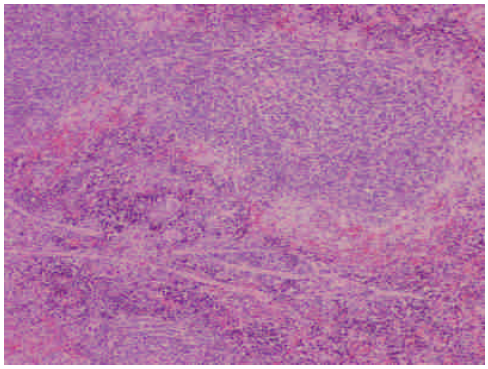
4x10
Relapsed tumor cell infiltrates in liver.



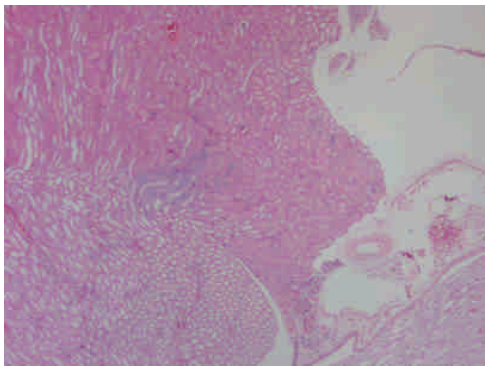
10x10
Relapsed tumor cell infiltrates in liver.



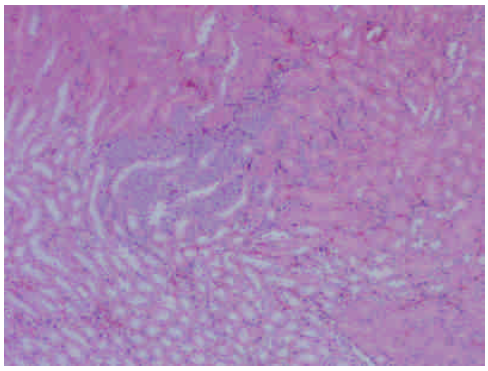
4x10
Relapsed tumor cell infiltrates in spleen.



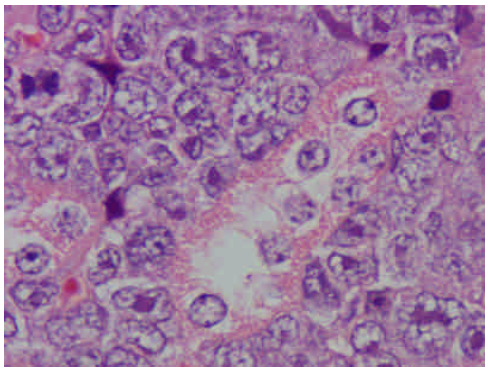
10x10
Relapsed tumor cell infiltrates in liver.



4x10
Relapsed tumor mass infiltrates in kidney.



10x10
Relapsed tumor mass infiltrates in kdney.



100x10
Relapsed tumor mass infiltrates in renal tubule.