Autologous Cytokine-Induced Killer Cells in the Treatment of Multiple Myeloma Concomitant with Lung Cancer and Paraneoplastic Dermatoses

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Abstract

Cytokine-induced killer (CIK) cells have been shown to be effective in the treatment of advanced cancer and minimal residual diseases. We report a multiple myeloma (MM) patient with concomitant lung cancer and paraneoplastic dermatoses, who received cellular immunotherapy with CIK cells which were derived from peripheral blood mononuclear cells (PBMCs) after being primed with anti-cluster of differentiation 3 (CD3) monoclonal antibody, interleukin-2 (IL-2), interferon-γ (IFN-γ) and IL-1. After treatment MM and lung cancer remained stable and no progression or recurrence was observed. Paraneoplastic dermatoses were obviously improved after treatment, which was first reported. No evident side effects were observed. These findings suggested that cellular immunotherapy with CIK cells was safe and effective in this patient with MM and lung cancer, and it might be a potent therapeutic option for paraneoplastic dermatoses.

Key words: cytokine induced killer cells, multiple myeloma, lung cancer, paraneoplastic dermatoses

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Introduction

Cytokine-induced killer (CIK) cells are a population of killer cells derived from peripheral blood lymphocytes after being primed with anti-cluster of differentiation 3 (CD3) monoclonal antibody, interleukin-2 (IL-2), interferon-γ (IFN-γ) and IL-1 (1). Although there are several different types of immune cell therapy such as IL-2-activated natural killer (NK) cells, lymphokine-activated killer (LAK) cells or tumor-infiltrating lymphocytes (TIL), CIK cells are superior to immune effector cells in removing minimal residual disease. The advantages of CIK cells include the following: 1) CIK cells exhibit non-major histocompatibility complex (MHC)-restricted killing of tumor cell targets both in vitro and in vivo (2, 3); 2) CIK cells are more easily expanded in vitro with a higher proliferation rate (3); 3) CIK cells posses higher antitumor cytotoxic activity (3); 4) CIK cells are capable of lysing a variety of tumor cell lines and fresh tumor isolates in vitro; 5) CIK cells do not require exogenous administration of IL-2 for in vivo activity; 6) CIK cells do not inhibit the growth of normal hematopoietic colonies (4); 7) The antitumor activity of CIK cells is not affected by cyclosporin or tacrolimus (5); 8) CIK cells are also sensitive to some multidrug-resistant tumor cells (6). Therefore as an adoptive cell immune therapy CIK cells are considered to be a promising method to remove minimal residual diseases.

Here, we report a case of multiple myeloma with lung cancer and paraneoplastic dermatoses, and the safety and efficacy of cellular immunotherapy with CIK cells were evaluated. After treatment, MM and lung cancer remained stable and concomitant paraneoplastic dermatoses were markedly improved in this patient.

Case Report

A 71-year-old man presented to our department. In 1999, his erythrocyte sedimentation rate (ESR; 23 mm/h) was found to be increased on routine examination, and thereafter, ESR ranged from 38 to 108 mm/h. In mid-March 2004, the
level of blood immunoglobulin (Ig) A was increased to 1,020 mg/dL. Lambda (λ) level was 1,010 mg/dL and IgM decreased. The levels of other lgs were in the normal range. Protein electrophoresis showed a monoclonal band in γ region. Immunofixation electrophoresis for IgA-λ was positive. Routine blood test, C reaction protein (CRP), kidney function, and serum blood calcium were normal. No positive findings were identified on positron emission tomography-computed tomography (PET-CT) scan. Bone marrow examination showed active proliferation of bone marrow and plasma cells accounted for about 9.2% of bone marrow cells, a fraction of which presented abnormal morphology. The patient was diagnosed as monoclonal gammopathy of undetermined significance (MGUS). Regular examination showed that IgA level was 973-1,010 mg/dL and λ level 373-739 mg/dL. In November 2008, bone marrow examination revealed that plasma cells accounted for about 13.6%.

Furthermore, magnetic resonance imaging (MRI) of thoracic vertebra showed an abnormal signal in T3 and T7. Then, this patient was diagnosed as asymptomatic multiple myeloma (MM). MM-related ostealgia, fever, anemia, renal failure or hypercalcemia was not presented. In May 2007, this patient was diagnosed as asymptomatic multiple myeloma with κ light chain. In the 18 months of follow-up period, immunofixation electrophoresis consistently showed IgA-λ and protein electrophoresis displayed a monoclonal band in γ region. The Ig levels were not dramatically changed. The ESR, and levels of albumin, β2-microglobulin, lactate dehydrogenase and creatinine remained relatively stable, and severe infection was not noted in the follow-up period. MRI of thoracic vertebra was normal after 6 months of cellular immunotherapy. New bone destruction was not found. Chest CT and PET-CT were also performed during follow-up and no new lesions were observed. Furthermore, detection of cancer markers including neuron-specific enolase, CEA, cytokeratin 19 fragment, carbohydrate antigen (CA) 72-4 and CA125 also were negative, which suggested no recurrence.

Then, peripheral blood mononuclear cells were isolated and primed with anti-CD3 monoclonal antibody, IFN-γ, and IL-2 and IL-1. After 14 days of culture, immunophenotype and survival rate of CIK cells were determined with a flow cytometer followed by transfusion of CIK cells. Furthermore, CIK cells were tested for killer activity on cell line 95D. The results showed when co-cultured with 95D cells for 48h CIK cells could kill more than 75% tumor cells at a ratio of effector to target cells of 1:10. This patient underwent 8 courses of cellular immunotherapy with CIK cells in combination with rhIL-2 treatment. One course of therapy was defined as follows: about 2-3×10⁷ of CIK cells (survival rate > 95%) were transfused twice and then rhIL-2 (100 wu daily) was subcutaneously administered for 10 consecutive days. Blood collection and CIK cell preparation were performed 2 weeks after last transfusion followed by another course of therapy. The interval between two courses of therapy was 1 month before the 8th course of therapy and then 2 months. Before therapy, the number of lymphocytes was 3.5±0.78×10⁹, which was expanded by 468±186.56 fold. Furthermore, the majority of CIK cells was positive for CD3 and CD8. The CD3+ cells and CD8+ cells accounted for more than 90% and 70%, respectively, after culture. The CD3+CD56+ cells were markedly increased and the CD4+ cells as well as CD3-CD56+ cells were profoundly decreased (Table 1). Figure 1 shows the results of flow cytometry.

Two weeks after CIK cell transfusion, peripheral blood was obtained for lymphocyte subset analysis. After cellular immunotherapy, the number and proportion of peripheral lymphocytes were increased over the course of therapy (Fig. 2 and 3). The numbers of CD3+ cells, NK cells or B lymphocytes were not markedly changed. The proportion of CD4+ cells was decreased from 37% to 25%, and that of CD8+ cells increased from 32% to 45%. Before the 8th course of therapy, the proportions of lymphocytes, CD3+ cells, CD8+ cells and CD3+CD56+ cells remained at the levels mentioned above, and 2 months after the 8th course of therapy, the proportions of the above lymphocyte subsets were decreased.

In the 18 months of follow-up period, immunofixation electrophoresis consistently showed IgA-λ and protein electrophoresis displayed a monoclonal band in γ region. The Ig levels were not dramatically changed. The ESR, and levels of albumin, β2-microglobulin, lactate dehydrogenase and creatinine remained relatively stable, and severe infection was not noted in the follow-up period. MRI of thoracic vertebra was normal after 6 months of cellular immunotherapy. New bone destruction was not found. Chest CT and PET-CT were also performed during follow-up and no new lesions were observed. Furthermore, detection of cancer markers including neuron-specific enolase, CEA, cytokeratin 19 fragment, carbohydrate antigen (CA) 72-4 and CA125 also were negative, which suggested no recurrence.

The patient had two types of cancer and cellular immunotherapy with CIK cells was performed to eliminate the residual tumor. Informed consent was obtained before therapy. Heparin anti-coagulated blood (50 ml) was collected followed by culture for expansion in a laminar flow cabinet.
Table 1. Immunophenotypes of Cytokine-induced Killer (CIK) Cells before and after Culture

<table>
<thead>
<tr>
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<th>Before culture (%)</th>
<th>After culture (%)</th>
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<tr>
<td>CD3+CD4-</td>
<td>37.42±5.67</td>
<td>86.33±4.39*</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>36.07±4.93</td>
<td>81.62±6.88*</td>
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<tr>
<td>CD3+CD56+</td>
<td>0.88±0.69</td>
<td>4.56±1.98*</td>
</tr>
<tr>
<td>CD8+CD56-</td>
<td>32.46±6.26</td>
<td>77.2±5.85*</td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>22.88±5.53</td>
<td>11.65±2.83*</td>
</tr>
<tr>
<td>CD3-CD56+</td>
<td>25.61±16.35</td>
<td>0.24±0.05*</td>
</tr>
<tr>
<td>CD8+/CD4+</td>
<td>1.63±0.35</td>
<td>7.43±2.2*</td>
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Note: CIK cells were prepared by incubation of peripheral blood monocytes in the presence of various types of cytokines including anti-CD3 monoclonal antibody, IL-2, IL-1 and γ -IFN. * represent P<0.05 vs before culture.

Figure 1. One of the representative immunophenotypes of CIK cells in flow cytometry. A: Immunophenotypes before culture. B: Immunophenotypes after culture. After culture, the CD3+CD8+ cells and CD3+CD56+ cells were increased from 33.09% to 86.23%, and 1.71% to 6.99%, respectively.

Desquamation and itching were improved. After 2 courses of therapy, symptoms except for mild desquamation disappeared. Skin lesions were completely improved after 5 courses of cellular immunotherapy, and desquamation, itching, rhagade or pain were not noted. But scattered pigmentation could be found. The quality of life was markedly improved. The cellular immunotherapy remains effective for about 10 months (Fig. 4). However, cellular immunotherapy was not performed within 3 months after 8 courses of cellular immunotherapy, and skin lesions re-occurred. Therefore, the long-term efficacy of cellular immunotherapy should be further investigated.

Discomfort was not observed during or after CIK cell transfusion. During rhIL-2 treatment, the patient complained of mild malaise and low-grade fever which were resolved after symptomatic treatment. In addition, clinical chemical
examination and routine blood test were performed at different time points before and after CIK cell transfusion. The results suggested that CIK cells did not significantly affect the routine blood test, liver and kidney function, cardiac enzymes, electrolytes and routine urine test.

Discussion

CIK cells are derived from peripheral lymphocytes after being primed with anti-CD3 antibody, IFN-γ, IL-2 and IL-1, and have antitumor activity (1). CIK cells are characterized by rapid proliferation, potent anti-cancer capability, wide anti-neoplastic spectrum, and sensitivity to multidrug-resistance cells. CIK cells can kill cancer cells in three ways: CIK cells directly kill the cancer cells; the activated CIK cells secret numerous cytokines which can kill cancer cells; the CIK cells regulate immune response directly killing cancer cells. Adoptive cellular immunotherapy treatment with CIK cells showed effectiveness in a variety of cancers including acute leukemia (7, 8), liver cancer (9, 10), lung cancer (11), gastric cancer (12) and prostate cancer (13). In addition, effectiveness of CIK cell transfusion has been observed in numerous hematological malignancies such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia and B-cell lymphoma (7, 14, 15), but only a few studies reported effectiveness in MM. An in vitro study showed CIK cells exerted cytotoxic effect on OPM-2 cells, a MM cell line, and this effect was enhanced in the presence of dendritic cells (16). Shi et al applied CIK cells in the treatment of two patients with MM, and recurrence was not found during the 2-year follow-up (17). An in vitro study and animal experiment also revealed the effectiveness of cellular immunotherapy with CIK cells in lung cancer (11). Clinically, cellular immunotherapy with CIK cells has been performed in lung cancer patients post-operation and was shown to promote the recovery of immune function (18). In addition, the efficacy of CIK cells in combination with chemotherapy in advanced non-small-cell lung cancer was superior to that of chemotherapy alone (19).

Tumor immunological studies show that cellular immunity of cancer patients is closely related to the occurrence and development of cancers. In MM patients, both B and T cell immunity are compromised. The number of peripheral lymphocytes of MM patients is decreased accompanied by a decreased proportion of CD3+ cells and CD4+ cells. On the contrary, the percentage of CD8+ cells is increased (20). The changes in the proportion of CD3+ cells, CD4+ cells and CD8+ cells in lung cancer patients are similar to those in MM patients (18). The increased proportion of CD8+ cells is regarded as a compensatory response which can suppress the proliferation of cancer cells. Perri et al showed in MM patients that the functions of helper T cells (Th cells) were normal, but the ability of suppressor T cells (Ts cells) to suppress B cells was enhanced. In addition, the B cells were more sensitive to Ts cells in MM patients (21). The immunopathogenesis of MM and lung cancer is the basis for cellular immunotherapy with CIK cells.

In the present report, the patient had multiple cancers (MM and lung cancer) concomitant with paraneoplastic dermatoses. MM was at the early stage and we postulated that MM was developed from MGUS. The lung cancer was classified as stage IA, and major tumor lesions were removed by surgery. The low tumor burden was beneficial for the cellular immunotherapy. Currently, chemotherapy is not recommended for asymptomatic MM patients. In addition, chemotherapy and radiotherapy are also not alternatives for patients with stage IA lung cancer and receiving surgery. To eliminate the residual lesions, cellular immunotherapy with CIK cells was performed in the present report.

In the present report, PBMCs were obtained from MM patients and used to prepare CIK cells, the quality and quantity of which met the clinical requirement. The majority of CIK cells was positive for CD3 and CD8, and CD3+CD56+ cells were markedly increased after culture. But the proportion of CD3+CD56+ cells was no more than 10%. After CIK cell transfusion, mental status was improved. A lot of parameters related to MM (such as Ig) and lung cancer were monitored, and results showed the disease remained stable and no recurrence was observed. For the present patient, the duration of follow-up was relatively short and the long-term efficacy of cellular immunotherapy should be further studied. Cellular immunotherapy with CIK cells was affected by the disease status, and the immune system was not severely disturbed at an early stage of cancer. Therefore, the immune
response of these patients is favorable and the efficacy will be subsequently enhanced. Therefore, for patients with plasma cell disease at an early stage such as MGUS, we postulate that CIK cells can regulate immune function and prevent it from developing into MM.

In the treatment of MM and lung cancer, skin lesions were markedly improved after CIK cells transfusion. For this patient, skin lesions occurred in MM and were worsened by lung cancer. Therefore, we postulated that skin lesions were associated with the abnormal immune response induced by MM and lung cancer, and then classified as a feature of paraneoplastic syndrome. At present, little is known about the pathogenesis of paraneoplastic dermatoses. The improvement in skin lesions might be explained by the following: 1) CIK cells killed residual cancer cells, which could secrete some cytokines resulting in skin lesions. With removal of residual diseases, cytokines from cancer cells decreased, which lead to improved skin lesions; 2) CIK cells could directly regulate the immune response. Currently, it is proposed that paraneoplastic syndrome is related to autoimmunity. CIK cells could regulate abnormal immune functions and subsequently improve skin lesions. This is the first study reporting that skin lesions in such patients can be improved by cellular immunotherapy with CIK cells. In future studies, the potential mechanisms underlying the therapeutic effects of CIK cells in such patients should be investigated.

Taken together, in this patient with MM, lung cancer and paraneoplastic dermatoses, CIK cells were prepared after in vitro priming, and transfused into this patient. Side effects related to CIK cell transfusion were few and two types of cancers remained stable during cellular immunotherapy accompanied by markedly improved paraneoplastic dermatoses.

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References


