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BRIEF REPORT
Long-Term Remission Induced by Corticosteroids, Cyclophosphamide, and Methotrexate in a Patient With Natural Killer Cell Leukemia

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Natural killer (NK) cell leukemia is a rare disease, and its prognosis is dismal. A standard therapy has not yet been established. Most patients die very soon (1–2 months) after the diagnosis according to the literature [1]. An innovative treatment strategy is warranted. Recently, there have been reports describing the efficacy of bone marrow transplantation [2,3]. It may become standard therapy if more experience with the method confirms these early reports. Meanwhile, our management of a girl with NK cell leukemia may be instructive. She had a long survival of 25 months with chemotherapy consisting of a combination of corticosteroids (CS), cyclophosphamide (CPM), and methotrexate (MTX). We also report the results of an in vitro drug sensitivity assay using a recently established bone marrow stroma-supported culture method.

Our patient was a 14-year-old girl who was admitted in March, 1994, because of a huge spleen. After splenectomy, her peripheral leukocytes gradually increased to a maximum of 100×10^9/liter. Most peripheral blood cells were large granular lymphocytes, and they were positive for CD2, CD16, and CD56 and negative for CD3 and CD57. NK activity was elevated. T-cell receptor genes were not rearranged, and Southern blotting using the Epstein Barr (EB) virus terminal probe showed two distinct bands. It suggested that NK cells might have originated either from one cell with two EB virus genomes integrated at the same time or from two clones with EB virus genome integrated in each clone. Because the intensity of two bands was almost identical, it is likely that NK cells might derive from one clone. The X-chromosome inactivation assay revealed that these cells were monoclonal, and NK cell leukemia was diagnosed. Hepatomegaly was noticed and her bone marrow had 37% of NK cells with apparently normal trilineage hematopoietic cells. The cells expressed p-glycoprotein detected with MRK16 antibody.

Leukapheresis was carried out and 10^{10} cells were eliminated; however, the NK cells recovered within 20 hr. Chemotherapy consisting of prednisolone, MTX, and cytarabine was initiated in October, 1994. The NK cell count decreased below 10×10^9/liter and it increased to over 30×10^9/liter after 2 weeks. Then we stopped using drugs that were shown to be ineffective by the in vitro assay. High-dose methyl-prednisolone, which was demonstrated to be effective in vitro, was initiated, along with high-dose CPM, which is not known to interact with p-glycoprotein. A remission was thereby induced. She continued receiving monthly CPM 1,000 mg/m^2 and weekly MTX, 15 mg/m^2. An HLA-identical donor was not available, and she refused bone marrow from alternative donors. She went to school without any trouble for 18 months, being hospitalized only to receive CPM for 2 days per month.

The disease recurred in the skin of her left leg in July, 1996, and subsequently relapsed in the central nervous system. In November, 1996, she died of sepsis from myelosuppression after chemotherapy containing dexamethasone, CPM, and MTX. An autopsy revealed that she had active disease in her bone marrow and pancreas. Southern blots of the DNA extracted from the pancreas showed a configuration identical to that of diagnostic peripheral NK cells using the EB virus terminal probe. The total duration of the disease after the diagnosis of NK cell leukemia was 25 months.

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TABLE I. Culture of NK Leukemic Cells on Bone Marrow Stroma

Control (no drug added)	100%[a]
Hydrocortisone	
1 μM	20%
10 μM	21%
100 μM	17%
Cytarabine	
0.1 μM	No reduction
1 μM	No reduction
10 μM	No reduction
Mitoxantrone	
0.1 μM	No reduction
1 μM	No reduction
10 μM	23% (Stroma damaged)

[a]Relative cell recovery after 7 day culture is shown.

RESULTS OF IN VITRO DRUG SENSITIVITY ASSAY

Previously, we established a bone marrow stroma-supported culture for acute lymphoblastic leukemia cells [4]. We applied this system to test the drug sensitivity of NK leukemic cells from this patient. The NK leukemic cells were seeded on allogeneic stromal layers and cultured for 7 days. In the control wells without cytotoxic drugs, most cells looked round, and they were viable and remained $CD3^-$ and $CD56^+$ assessed by flow cytometry. Table I shows the result of the drug sensitivity assay. The NK leukemic cells were very resistant to cytotoxic agents such as cytarabine and mitoxantrone, whereas the cells were sensitive to hydrocortisone.

DISCUSSION

Several points can be made from our experience with this patient with NK cell leukemia. First, the disease responded to steroids and cyclophosphamide very well. It has been reported that NK cells express p-glycoprotein [5]. Such cells are resistant to cytotoxic drugs, such as anthracyclines, epipodophylotoxins, and vinca alkaloids. Agents that do not interact with p-glycoprotein, such as steroids and alkylating agents, may be the key drugs in NK cell leukemia. Second, the combination of CPM and MTX was effective in maintaining a good hematologic and performance status for as long as 18 months. If transplantation is planned for patients with NK cell leukemia, we recommend using this combination of drugs to maintain remission until the transplantation is performed. Third, the in vitro assay previously established for acute lymphoblastic leukemia [4] could also be applied to NK cell leukemia. The lack of data on the efficacy of the drugs for NK cell leukemia is also due to the lack of a suitable culture system for NK leukemia cells. Based on the in vitro data we could avoid ineffective drugs in this patient, such as cytarabine and mitoxantrone, that are widely used for aggressive leukemias and lymphoma. The accumulation of in vitro data will be useful for formulating a development of new therapeutic regimens for NK cell leukemia. Finally, we propose a prospective trial for NK cell leukemia employing the combination of drugs that induced a long term remission in the patient described in this report.

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