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Original Article

In Vitro Effects of Recombinant Human IL-11 on Human Malignant Lymphoma Cells

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IL-11 is a cytokine considered to have potential for the treatment of thrombocytopenia patients undergoing chemotherapy. The administered dose of IL-11 must not facilitate growth of malignant cells in addition to its thrombopoietic effect. In this study we focused on malignant lymphoma (ML), in which thrombocytopenia is a common adverse effect of chemotherapy. Cells obtained from biopsied lymph nodes of 7 patients were cultured with and without recombinant human IL-11 (rhIL-11). Cells growth was analyzed by the MTT assay. rhIL-11 did not enhance the proliferation of the lymphoma cells at any concentration. We believe that rhIL-11 is an expected agent for ML patients by the potential to reduce chemotherapy-induced thrombocytopenia.
Key words malignant lymphoma, proliferation, recombinant human IL-11

INTRODUCTION

Interleukin 11 (IL-11) is a cytokine that was identified in conditioned media of a immortalized primate bone marrow stromal cell line[1] and cloned from a human fetal lung fibroblast cell line[2]. IL-11 enhanced blast colony formation *in vitro*, indicating its effect on primitive progenitor cells[3]. rhIL-11 also has direct effects on megakaryocytes and its precursors *in vitro*[4]. Among these, the effects on megakaryocytopoiesis are of special interest. IL-11 acts synergistically with IL-3 to stimulate various stages of megakaryocytopoiesis and thrombopoiesis in human bone marrow (BM) cells[5,6]. Based on *in vitro* effects, rhIL-11 has been used in patients with chemotherapy-induced thrombocytopenia. IL-11 has significantly reduced chemotherapy-related morbidity and mortality[7–11], and is associated with accelerated recovery of both hematopoiesis and the immune system[12].

IL-11 is also a B lymphocyte activator. In combination with stem cell factor (SCF) or IL-4, IL-11 supports the generation of B cells from the bone marrow of 5-fluorouracil (5-FU)-treated mice[13]. IL-11 and IL-4 can also reverse the inhibitory effect of IL-3 on early B-lymphocyte development[14]. The promotion of B-cell differentiation may be mediated by T cells[15,16]. IL-11 appears to act on hematopoietic stem cells in a manner similar to IL-6. The IL-11 receptor (IL-11R) complex consists of the ligand binding α chain (IL-11Rα) and the signal transducing subunit, gp130[17]. IL-11 shares gp130 with IL-6, LIF, Oncostatin M and CNTF, which results in partly overlapping activities[16,18–20]. Because IL-6 is known to promote differentiation of human B cells[21–24], it is possible that IL-11 may also play a role in human B-cell maturation. IL-11 stimulated the growth of the IL-6-dependent murine plasmacytoma cell line (T1165). Furthermore, IL-11 enhances formation of immunoglobulin (Ig)-secreting B cells in a standard murine spleen cell plaque-formation assay[1].

When IL-11 is used to treat thrombocytopenia patients, especially those with

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malignant lymphomas, its potential to activate malignant cells should be considered. It is not clear, however, whether lymphoma cells are stimulated by IL-11. In this study we investigated the effect of rhIL-11 on proliferation of fresh lymphoma cells using an *in vitro* culture system.

MATERIALS AND METHODS

Patients' characteristics

Seven consecutive patients with malignant lymphoma were included in this study after giving informed consent. Lymph nodes (LN) of these patients were biopsied for routine diagnosis. Portions of the LN were also processed for the investigative assay. The characteristics of these patients are summarized in Table 1. Patients ranged in age from 30 to 89 y. There were 4 males and 3 females. Pathological diagnosis was made according to the Revised European-American Classification of Lymphoid Neoplasms (REAL)[25].

The surface phenotype was analyzed by indirect immunofluorescence assay, using flow cytometry (FACScan ; Becton Dickinson Immunocytometry Systems, San Jose, CA, USA).

Culture conditions

Cell suspensions were prepared from biopsied specimens. Adhered cells were removed by incubating for 1 hour at 37°C in RPMI1640 (SIGMA, U. S. A.) medium supplemented with 20% fetal calf serum (FCS ; EQITECH-BIO, INC., U. S. A.) in culture flasks. The viability of nonadherent cells was assessed by trypan blue dye for which exclusion was always greater than 90%. Cells were seeded at $1-2\times10^6$ cells/ml in RPMI1640 medium supplemented with 20% FCS with the presence or absence of cytokines, and incubated for 4 to 5 days at 37°C in 5% CO_2. The recombinant human (rh) IL-11 was a generous gift from Yamanouchi Pharmaceutical (Tokyo, Japan). The final concentrations of rhIL-11 in culture media were 0, 1, 10 and 100 ng/ml.

To test the proliferative activity of cells from biopsied lymph nodes in response to a cytokine we added rhIL-2 (Yamanouchi Pharmaceutical, Tokyo, Japan) to each experiment as controls. The final concentrations of rhIL-2 in culture madia were 0, 5, and 10 ng/ml. The *in vitro* sensitivity of lymphoma cells to rhIL-11 and rhIL-2 was determined using the tetrazolium-based colorimetric assay (MTT assay)[26].

Single-cell suspensions were seeded at 1×10^5 cells/ml in 96-well culture plates. After 4 or 5 days incubation of lymph node cells with rhIL-11 or rhIL-2, MTT solution (SIGMA, U. S. A.) was added to each well of the culture plate as it's final concentration was 500μg/ml. After 3 or 4 hours of incubation at 37°C with MTT, the unreacted MTT and medium were removed and dimethyl sulfoxide (DMSO) was added to solubilize the MTT formazan. The OD of each well was measured with a microplate spectrophotometer (WAKO, Japan) equipped with a 540 nm filter. Relative MTT is the MTT value of each cytokine concentration divided by a control MTT value without cytokines. Each experiment was performed in triplicate. Statistical significance was determined using Dunnett's multiple comparison test.

RESULTS

Seven consecutive patients with ML were included in this study. There were two cases of

Table 1. Clinicopathological features

Case No.	Age (yr)	Sex	Pathological diagnosis	% of malignant cells
1	54	M	Diffuse large B-cell lymphoma	61.2
2	88	M	Hodgkin's disease (Mixed cellularity)	NT
3	76	F	Diffuse large B-cell lymphoma	28.8
4	64	F	Diffuse large B-cell lymphoma	85.5
5	57	M	Angioimmunoblastic T-cell lymphoma	52.8
6	30	F	Peripheral T-cell lymphoma, unspecified	48.8
7	89	M	Hodgkin's disease (Mixed cellularity)	NT

% of malignant cells indicates the proportion of lymphoma cells in samples judged by FACS analyses, as described in Materials and Methods. NT : not tested.

Hodgkin's disease (HD), three diffuse large B-cell lymphoma (DLBL), one peripheral T-cell lymphoma (PTCL) and one angioimmunoblastic T-cell lymphoma (AILD) (Table 1). Experiments were performed using cells obtained from biopsied lymph nodes. Since the lymph nodes carried both malignant and non-malignant cell, percentages of malignant cell populations were assessed by FACS analysis. In general, as shown in Table 1, half or more of the cells were in the malignant cell population. For lymph nodes from HD we did not conduct a FACS analysis because only small percentages of cells were considered to be derived from a malignant cell population.

To test the effect of rhIL-11 on the lymphoma cells, the cells were cultured for 4 to 5 days in the presence of rhIL-11 at four different concentrations: 0, 1, 10 and 100 ng/ml. Subsequently, cells were harvested to assess growth by the MTT assay. Results of the MTT assay are shown in Fig. 1. Six cases [UPN 1 (DLBCL), 2 (HD), 3 (DLBCL), 5 (AILD), 6 (PTCL) and 7 (HD)] showed no significant proliferation of cells in response to the addition of rhIL-11 at various concentrations. In UPN 4, a significant increase was observed at 10 ng/ml of rhIL-11, but addition

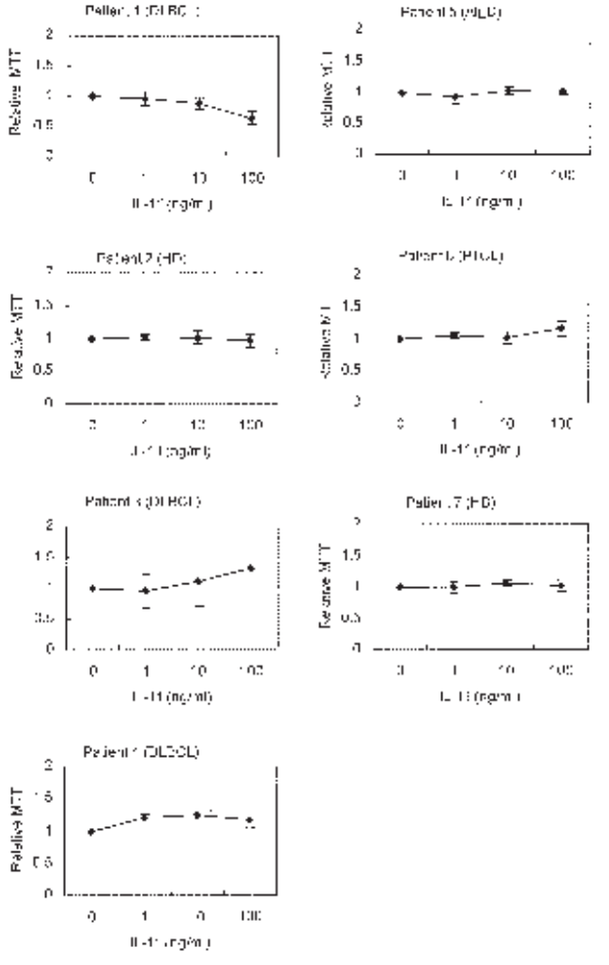

Fig. 1. Effect of rhIL-11 on lymphoma cell proliferation. Lymphoma cells from each patient were cultured with rhIL-11 for 4–5 days. The final concentrations of rhIL-11 are 0, 1, 10 and 100 ng/ml. Relative MTT shows the value of each concentration divided by the control value. The error bars reflect the S. D. for each experimental circumstance, indicated as each value relative to the results for cells in culture without rhIL-11. *P<0.05.

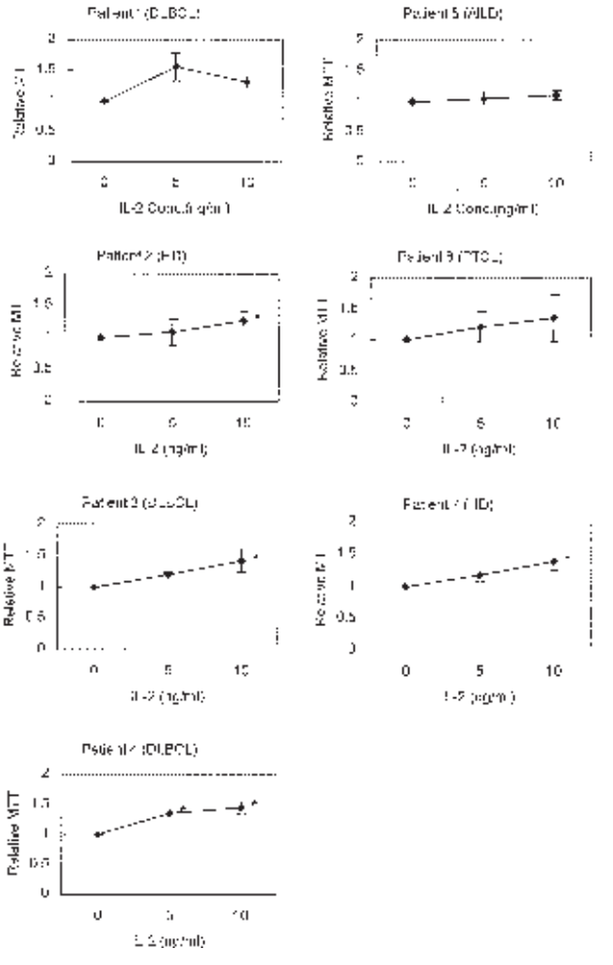

Fig. 2. Effect of rhIL-2 on lymphoma cell proliferation. Lymphoma cells from each patient were cultured with rhIL-2 for 4–5 days. The final concentrations of rhIL-2 are 0, 5 and 10 ng/ml. Relative MTT shows the value of each concentration divided by the control value. The error bars reflect the S. D. for each experimental circumstance, indicated as each value relative to the results for cells in culture without rhIL-2. *P<0.05.

of 100 ng/ml of rhIL-11 did not show any significant stimulation in UPN 4. These results should be contrasted to those using rhIL-2 instead of rhIL-11 (Fig. 2). In all cases the lymph node cells proliferated in response to the addition of rhIL-2. Furthermore, these increases were statistically significant in most of the cases, as indicated by asterisks in Fig. 2.

To test the effectiveness of the rhIL-11 used in this study, we cultured TF-1 cells, a human myeloid line, with or without the rhIL-11. It has been known that TF-1 cell proliferation can be stimulated with IL-11[16]. The growth of TF-1 cells were significantly stimulated by the addition of IL-11 at a concentration of 100 ng/ml ($p < .05$) (data not shown). These results demonstrated that the refractoriness of the lymph node cells to the rhIL-11 in most of the cases was not due to either poor culture condition or low viability of the cells.

DISCUSSION

This study tested the safety of IL-11 for tumor cell proliferation in patients with malignant lymphoma (ML). Because IL-11 functions as a growth and a differentiating factor of B lymphocytes[14], it is reasonable to be concerned that IL-11, when administrated to ML patients, may enhance tumor growth. To address this issue we examined the *in vitro* effect of IL-11 on proliferation of lymphoma cells obtained from patients with ML. rhIL-11 showed no significant effects in six out of seven cases (Fig. 1). Because IL-2 stimulated the growth of the lymph node cells *in vitro*, in our experiment, the cells were sufficiently viable to respond to some cytokines (Fig. 2). Also sufficient was the activity of the rhIL-11 used, as the cytokine stimulated the growth of TF-1 cells that expressed the IL-11 receptor (IL-11R) on their surface. These results suggest that in most patients with ML, rhIL-11 would not stimulate the growth of the tumor.

In UPN 4, the cells seem to proliferate in the presence of rhIL-11 (Fig. 1). In this case, 10 ng/ml of rhIL-11 stimulated cell proliferation significantly, although 100 ng/ml did not. Although our results suggest that the lymphoma cells from UPN 4 could partially respond to rhIL-11. This seems to be less likely because no dose-dependent response was observed at the higher, 100 ng/ml, concentration. Because we could only analyze a small number of patients we could not exclude the possibility that lymph node cells from some minor population of the patients were able to respond to rhIL-11. Examining of larger numbers of lymphoma patients would clarify this point.

Based on our results, we would describe the role of normal (non-malignant) cells on the growth of lymphoma lesions treated by rhIL-11. Two patients with HD were included in this study (UPN 2 and 7, see Table 1). The lymphoma lesion of this type of ML is, in general, largely composed of non-malignant cells. Moreover, in most patients other than HD in this study, approximately 50% or more cells were deemed to be non-malignant (Table 1). The addition of rhIL-11 did not enhance the lymph node cell proliferation in these cases (Fig. 1). Therefore, it would be at least possible that, among the cellular components of LN, the non-malignant components (except adherent cells during *in vitro* culture) also could not respond to rhIL-11 *in vitro*.

The human IL-11 receptor (IL-11R) α is expressed in myeloid (K562[27], HL60[28]), megakaryocytic (Mo7E[12]), and erythroid (TF-1[27]) leukemia cell lines. The activity of the rhIL-11 strain used was sufficient, as the cytokine stimulated the growth of TF-1 cells that express IL-11R on the surface. But its expression on lymphoma cells remains to be elucidated. In this study, we examined the expression of IL11R α on these lymphoma cells by flow cytometry and immunohistochemical staining. Unfortunately, we could not detect any specific signal for IL-11R α by these methods because of some non-specific reaction of the antibody (data not shown).

Chemotherapy-induced thrombocytopenia has been an increasingly common problem among oncology patients. Until now, the only way to treat the thrombocytopenia patients has been by transfusion of platelet concentrates donated by healthy volunteers. There are a few problems related to platelet transfusion, in particular, the risk of viral transmission and resistance to platelet transfusion that occurs in some patients requiring frequent platelet transfusions[29,30,31]. rhIL-11 was used safely in patients with severe chemotherapy-induced thrombocytopenia and rhIL-11 treatment at a dose of 50 μg/kg allowed a significant number of such patients to avoid platelet transfusions during a subsequent chemotherapy cycle[32].

In pharmacokinetic data on healthy male

volunteers after single subcutaneous (s. c.) injection of 3 μg/kg to 50 μg/kg, the peak plasma rhIL-11 concentrations (C_{max}) were between 0.9 and 19.0 ng/ml[33]. Our excess concentrations, even 100 ng/ml in this study, did not stimulate lymphoma cell proliferation. Thus, introduction of rhIL-11 into the treatment of chemotherapy-induced thrombocytopenia in lymphoma patients might have some benefit for patients if rhIL-11 does not exacerbate lymphoma lesions. Our results indicate that rhIL-11 cannot support *in vitro* growth of lymphoma cells in most patients with ML, suggesting that rhIL-11 does not stimulate the growth of lymphoma cells *in vivo*. Thus, rhIL-11 may be used in the treatment of thrombocytopenia after chemotherapy with malignant lymphoma patients to stimulatory effects on lymphoma cells *in vivo*.

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