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Preclinical paper

Recombinant human interleukin-11 is unlikely to stimulate the growth of the most common solid tumors

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Recombinant human interleukin-11 (rhIL-11) has been shown to enhance recovery from thrombocytopenia and mucosal injury after cancer chemotherapy. Since RNA for the receptor and signal transducer for IL-11 is detected in many cell types including some cancer cells, it was theoretically possible that rhIL-11 could affect the growth of tumor cells. This study was intended to determine whether rhIL-11 stimulates the proliferation of human tumor colony-forming units (TCFUs) taken directly from patients. Tumor cells were cultured in soft agar and continuously exposed to three concentrations of rhIL-11 (1.0, 10.0 and 100.0 U/ml) for 14 days in the capillary cloning system. Growth stimulation was noted in two of 66 (3%) of evaluable specimens, including one of 14 evaluable non-small cell lung cancer and one of five evaluable colon cancer specimens. In these two specimens, there was no increased stimulation of TCFUs with escalating concentrations of rhIL-11. Interestingly, the highest concentration of rhIL-11 tested inhibited the growth of 16 specimens (24.2%; 95% confidence interval 13.9–34.5%). Growth inhibition demonstrated a concentration–response relationship ($p < 0.001$). These results suggest that rhIL-11 appears unlikely to stimulate the growth of the most common solid tumors. [© **1999 Lippincott Williams & Wilkins.**]

Key words: Cytokine, interleukin-11, human tumor cloning assay.

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Introduction

Interleukin-11 (IL-11) is a stromal cell-derived cytokine which has multiple effects on hematopoietic and non-hematopoietic cells, including stimulation of megakaryocytopoiesis, neuronal differentiation, osteoclastogenesis and the inhibition of adipocyte differentiation.[1,2] Several studies have shown that recombinant human IL-11 (rhIL-11) can increase the production of platelets and decrease the need for platelet transfusion in patients receiving cancer chemotherapy.[3–5] Also, rhIL-11 has been reported to stimulate the recovery of epithelial cells of the intestine and oral mucosa injured by chemo- and radiotherapies in animal models.[6,7]

IL-11 belongs to a family of cytokines including interleukin-6, leukemia inhibitory factor, ciliated neurotrophic factor, oncostatin M and cardiotrophin which share a common signal-transducer, gp130.[1] IL-11 binds the α chain receptor on the cell surface (IL-11R) and then the IL-11-IL-11R complex activates gp130.[8] IL-11R α chain expression was reported not only in megakaryocytes, colon epithelium and osteoclasts but also breast cancers, colon cancers and melanoma.[9–11] In addition, gp130 expression is seen in most cancer cells.[12] Recent studies using cell lines have shown that rhIL-11 inhibits the growth of breast cancer cells,[9] acts in synergy with IL-3 to stimulate leukemia cell lines and can stimulate myeloma cell lines,[13–15] while it has no effect on the growth of fresh myeloma cells[15] or melanoma.[11] Since rhIL-11 could become a therapeutically important molecule in supportive care for patients who receive cancer chemotherapy, it is important to be certain that rhIL-11 does not stimulate growth of tumor cells. In this study, we evaluated the effect of

rhIL-11 on the proliferation of tumor cells taken directly from patients, using a human tumor colony-forming assay.[16,17] This approach may be more clinically relevant than using human tumor cell lines to evaluate the effects of IL-11 on proliferation of human tumor cells.

Materials and methods

rhIL-11 was supplied by Genetics Institute (Cambridge, MA). Stocks of rhIL-11 were made up in CMRL 1066 and frozen at −70°C. Dilutions with CMRL 1066 were made at the time of the experiment with CMRL 1066 serving as the control. The final concentrations of IL-11 tested were 1.0, 10.0 and 100.0 U/ml as a continuous exposure. A unit is defined as 0.5 ng rhIL-11. The highest concentration (100 U or 50 ng/ml) is representative of mean peak concentrations obtained with the s.c. clinical dose of 50 μg/kg.[18]

Tumor samples

After written informed consent was obtained in accordance with institutional and Federal guidelines, tumor specimens (biopsies, pleural effusions and ascites) were collected by sterile techniques as part of a diagnostic work-up or as part of treatment. No samples were obtained solely for research purposes. All samples were granted exempt research status by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

Solid tumors or lymph nodes were minced into 2-5 mm fragments in the operating room and immediately placed in McCoy's Medium 5A (Mediatech, Herndon, VA) plus 10 mM HEPES buffer (Sigma, St Louis, MO), 2 mM sodium pyruvate, 10% heat-inactivated newborn calf serum, 50 U/ml penicillin and 50 mg/ml streptomycin (all Gibco, Grand Island, NY). Within 4 h, these solid tumors were mechanically disassociated with scissors, forced through a no. 100 stainless steel mesh, sieved (Sigma) through a 25 gauge needle, and centrifuged at 1000 r.p.m for 7 min. Harvested cells were then washed with McCoys medium 5A, plus 20 mM HEPES buffer (Sigma), 10% fetal calf serum (Tissue Culture Biologicals, Tulare, CA), 2 mM sodium pyruvate, 50 U/ml penicillin and 50 mg/ml streptomycin (Gibco). Ascitic, pleural, pericardial fluids and bone marrows were obtained by standard techniques and placed in sterile containers. Then 10 U/ml preservative-free heparin (Sigma) was added immediately after collection of fluid or bone marrow to prevent coagulation. After centrifugation at 1000 r.p.m for 7 min, the cells were harvested and washed as above. The viability of cell suspensions was determined on a hemocytometer with Trypan blue.

Human tumor cloning assay

The human tumor capillary cloning assay was performed using the soft agar system as previously reported.[16,17] Cells to be cloned were suspended in 0.3% agar (Difco, Detroit, MI) in double-enriched CMRL 1066, supplemented with 50 U/ml penicillin, 50 mg/ml streptomycin, 0.3 mM ascorbic acid, 2 mM GlutaMAX-1, 0.6 mg/ml asparagine (all Gibco), 15% heat-inactivated horse serum (Sigma), 2 U/ml insulin (Sigma) and 2% heat-inactivated fetal calf serum (Tissue Culture Biologicals).

The concentrations of rhIL-11 were adjusted to 0.5, 5 and 50 ng/ml (1, 10 and 100 U/ml, respectively) in the cell suspension to allow for 14 day continuous exposure. The concentrations of rhIL-11 used have been demonstrated to stimulate the megakaryocytopoiesis *in vitro*.[19,20] Recent clinical trials have revealed that the optimal schedule of rhIL-11 is 50 μg/kg s.c. once daily for 14-21 days[3,4] and the peak plasma concentration of rhIL-11 is 19.1 ± 3.2 ng/ml.[18] The range of concentrations of rhIL-11 used in our study included the clinically relevant levels.

Utilizing capillary action, 100 ml of the mixture containing 20 000 cells was placed in each capillary tube. The ends of the capillary tube were sealed with clay. Six capillary tubes were prepared for each data point. The tubes were placed in a 7% CO_2 incubator at 37°C for 14 days. To determine the number of colonies in the tubes, the agar was extracted from the capillary tube onto a microscope slide by removing the clay. The number of colonies (defined as more than 50 cells) on each slide was then counted under a microscope.

Quality control

To assure the presence of an excellent single-cell suspension on the day of plating, positive and untreated controls were used in addition to the drug-treated tubes.[21] For each tumor tissue sample tested, six positive control tubes were set up to contain the cell poison orthosodium vanadate at 200 mg/ml. This positive control should destroy all clonogenic cells. Six

untreated control tubes were also set up on day 0. The tubes were placed in a 37°C incubator and were removed on day 14 for counting of the number of colonies in each tube. If there was no effect on colony formation, then the single-cell suspension on day 0 was poor (since orthosodium vanadate does not affect clumps), the tumor sample test was considered non-evaluable and excluded from the results.

Definition of an *in vitro* response and statistical analysis

A test is defined as an experiment, performed on a unique tumor tissue sample, that contains untreated control, positive control, and three specified compound concentration levels. An experiment was considered evaluable for analysis when the untreated control had ≥ 33 colonies/tube and the vanadium control had 30% or less colony formation of the untreated control. The inhibition and stimulation of colony formation was defined as 50% or less and 150% or greater of the untreated control, respectively. No effect was reported when the result was 51-149% of the untreated control. The overall effect of rhIL-11 on a set of tumor specimens was expressed as percentage and 95% confidence interval (95% CI). The dose-response relationship of rhIL-11 was evaluated by the Mantel extension test and a two-tailed $p < 0.05$ was considered to indicate statistical significance.

Table 1. Number of evaluable specimens of tested tumors in the capillary cloning system

Tumor type	Tested specimens	Evaluable specimens[a]
Breast	24	17 (70.8%)
Ovarian	19	15 (78.9%)
Non-small cell lung	27	14 (51.9%)
Colon	6	5 (83.3%)
Mesothelioma	4	3 (75.0%)
Brain	3	2 (66.7%)
Melanoma	2	2 (100%)
Gastric	3	2 (66.7%)
Others	18	6 (33.3%)
Total	106	66 (62.3%)

[a]Evaluable specimens were defined as those containing ≥ 33 colonies/tube and with vanadium controls containing $\leq 30\%$ of the number of colonies in the untreated control.

Results

Sixty-six out of 106 specimens (62.3%) were evaluable (Table 1). In the 40 non-evaluable specimens, 37 had inadequate colony numbers and three had unacceptable vanadium controls (specimens demonstrated cell clumping on day 0). The 66 evaluable specimens included 17 breast cancers, 15 ovarian cancers, 14 non-small cell lung cancers and five colon cancers (Table 1). Although growth stimulation was noted in one of 14 non-small cell lung cancers and one of five evaluable colon cancers for an overall rate of two of 66 (3.0%), no increased stimulation with escalating concentrations of rhIL-11 was observed (Table 2).

Importantly, rhIL-11 inhibited the growth of 16 of 66 specimens (24.2%, 95% CI 13.9-34.5%), including four of 17 breast cancers (23.5%, 95% CI 6.8-49.9%) and five of 14 non-small cell lung cancers (35.7%, 95% CI 12.8-64.9%). Growth inhibition by rhIL-11 demonstrated a concentration-response relationship ($p < 0.001$ by the Mantel extension test, Table 3).

Table 2. Number of evaluable tumors showing growth stimulation in the clonogenic assay at three concentrations of rhIL-11[a]

Tumor type	Evaluable specimens	Concentrations of rhIL-11 (U/ml)		
		1.0	10.0	100
Breast	17	0	0	0
Ovarian	15	0	0	0
Non-small lung	14	1 (7.1%)	1 (7.1%)	1 (7.1%)
Colon	5	1 (20.0%)	1 (20.0%)	0
Mesothelioma	3	0	0	0
Brain	2	0	0	0
Melanoma	2	0	0	0
Gastric	2	0	0	0
Others	6	0	0	0
Total	66	2 (3.0%)	2 (3.0%)	1 (1.5%)

[a]Stimulation is defined as 150% or greater survival.

Table 3. Number of evaluable tumors showing growth inhibition in the clonogenic assay at three concentrations of rhIL-11[a]

Tumor type	Evaluable specimens	Concentrations of rhIL-11 (U/ml)		
		1.0	10.0	100
Breast	17	0	0	4 (23.5%)
Ovarian	15	0	0	2 (13.3%)
Non-small lung	14	0	1 (7.1%)	5 (35.7%)
Colon	5	0	0	0
Mesothelioma	3	0	0	0
Brain	2	0	0	1 (50.0%)
Melanoma	2	0	1 (50.0%)	1 (50.0%)
Gastric	2	0	0	1 (50.0%)
Others[b]	6	0	1 (16.7%)	2 (33.3%)
Total	66	0	3 (4.5%)	16 (24.2%)

[a]Inhibition is defined as 50% or less survival. The concentration–response relationship was evaluated by the Mantel extension text ($p < 0.001$).
[b]Inhibition was observed in one of one carcinoid at 10 and 100 U/ml, and one of one prostate cancer at 100 U/ml.

Discussion

The endpoint of this study was to determine whether rhIL-11 has significant potential to stimulate growth of solid tumors. There are multiple examples where known mitogens have been documented to increase colony formation of tumors taken directly from patients when those tumors are grown in soft agar.[22-26] Therefore, if IL-11 is a mitogen for tumor cells in patients, the human tumor cloning system utilized should detect that stimulation.

A previous study to determine the effects of granulocyte macrophage colony stimulating factor (GM-CSF) on human tumors found that recombinant human GM-CSF did not cause stimulation of colony formation.[27] This *in vitro* finding was predictive for the clinical situation because GM-CSF has not been shown to promote tumor growth in any patients with solid tumors to date. The present study indicates that 97% of the specimens tested are not stimulated. In the two specimens which were stimulated, no dose-response was observed. In fact, of the non-small cell tumors, more exhibited growth inhibition (five of 14 versus one of 14).

Of note is that rhIL-11 inhibited the growth of 24.2% of the specimens; in particular, those of breast cancers and non-small-cell lung cancers. The mechanism by which this occurs is unknown.

There is no indication from clinical trials[3,28] that rhIL-11 stimulates the growth of non-hematologic malignancies. This is consistent with our study in which there was no clear relationship between rhIL-11 and growth stimulation of many common solid tumors. This is in contrast to IL-6, a factor which shares the same signal transducer as IL-11 but is considered an autocrine or paracrine growth factor for some cancers.[29-31] The differences between IL-6 and Il-11 may be explained by differential expression of the respective α chains required for cytokine binding.

In conclusion, our results suggest that rhIL-11 is unlikely to stimulate the growth of most common solid tumors and continued observations in clinical trials would be worthwhile.

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