F1t3 RECEPTOR EXPRESSION ON THE SURFACE OF MALIGNANT HEMATOPOIETIC CELLS AND RESPONSES TO F1t3 LIGAND STIMULATION

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ABSTRACT

Objective: To investigate the F1t3 receptor expression on the surface of malignant hematopoietic cells, the effect of TNFα and dexamethasone (DXM) on its expression and the responses of those cells to recombinant human F1t3 ligand (rhFL). Methods: Eighteen malignant hematopoietic cell lines were determined for the F1t3 receptor expression by flow cytometric analysis. The effect of rhFL on the proliferation of malignant hematopoietic cells *in vitro* was measured using MTT assay. Results: The expressions of F1t3 receptor on the surface of Raji, Daudi, HL-60, 8266 and XG-6 cells were detected by flow cytometric analysis. Following incubation with 20 ng/ml TNFα for 24h, the number of F1t3 receptor positive cells decreased in Raji and 8266, increased in HL-60 and XG-6, and no difference in Daudi cells. After incubation with 10^{-6} mol/L DXM for 24h, the number of F1t3 receptor positive cells decreased in all the 5 F1t3 receptor positive cell lines. rhFL stimulated the proliferation of HL-60 and Raji cells. Conclusion: For most of the malignant hematopoietic cells, there was neither the expression of F1t3 receptor nor the response to rhFL. DXM may be useful to reduce the effect of FL on the proliferation of some F1t3 receptor positive malignant hematopoietic cells in vitro and *in vivo*.

Key words: F1t3 receptor; Recombinant human F1t3 ligand (rhFL); Malignant hematopoietic cell lines; Proliferation; Dexamethasone (DXM)

Hematopoiesis is a complex process in which cell growth and differentiation are controlled by a number of hematopoietic growth factors or cytokines, acting as either positive or negative regulators. Disruption of balanced hematopoiesis may lead to the uncontrolled clonal expansion of a cell lineage, causing the leukemic transformation. Among them, class III receptor-type tyrosine kinases (RTKs) and their ligands seem to play an important role in hematopoiesis, especially in the early stages of the process.[1] F1t3 receptor, fms-like tyrosine kinase receptor III, was first identified by Lyman in 1993. F1t3 receptor is expressed in adult bone marrow and thymus, fetal thymus and fetal liver. F1t3 ligand (FL) is a hematopoietic growth factor that induces the proliferation and survival of primitive hematopoietic progenitor and stem cells.[2] FL may also stimulate the production of dendritic and natural killer cells, both in vitro and in vivo. Thus FL may find uses in the stimulation of hematopoiesis and in cancer therapy.[3-5]

However, many of the malignant hematopoietic cells derive from the transformation of hematopoietic stem/progenitor cells. It may be reasonable to reveal the role played by FL and its receptor in the proliferation of those malignant cells. An investigation of potential effects of FL on malignant hematopoietic cells may be relevant to a clinical usage of the cytokine as a antitumor biotherapy factor or as a hematopoietic factor in dyshematopoiesis of the patients. In this report, we investigated the pattern of F1t3 receptor expression in leukemia cell lines and the effects of TNFα and DXM on its expression. The mitogenic potential of rhFL for the proliferation and colonial growth of leukemia cell lines was also evaluated.

MATERIALS AND METHODS

Cytokines and Antibody

Recombinant human FL (rhFL) was expressed

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with a yeast expression system in our department.[6] Dexamethasone (DXM) was obtained from Sigma. IL-6, GM-CSF, TNFα and IL-11 were purchased from Immugenex (Los Angeles, USA). Anti-Flt3- specific IgG1 monoclonal antibody SF1.340 was purchased from Immunotech (Marseille, France).

Cell Lines and Their Cultures

Jurkat, Daudi, Raji, HL-60, U937, KG1-α, U266, TF1, Baf, 8266 and Wehi cell lines were from the ATCC, USA; the B9 cell line was donated by Dr. L. Arden, Amsterdam; B9-11, XG-1, XG-6 and XG-7 cell lines were a gift from Dr. B. Klein, Montpellier, France. HSB2, JJhan and Sub T1 cell lines were kindly provided by Professor Yao Kun, Nanjing Medical University, China. All cell lines in our laboratory were free from mycoplasma contamination. Cell lines were grown and maintained under routine conditions (37°C in a humidified atmosphere with 5% CO_2) in RPMI 1640 supplemented with 10% inactivated fetal bovine serum. 1.5% Wehi supernatant was added as a source of IL-3, required for the growth of the Baf cells. Ten ng/ml of GM-CSF, 1ng/ml of IL-6 and 0.5ng/ml of IL-11 were also added to TF1; XG-1, XG-6, XG-7 and B9-11 (B9) cell lines, respectively. In order to investigate the effect of TNFα and DXM on the expression of Flt3 receptor on the surface of malignant hematopoietic cells, 20 ng/ml of TNFα or 10^{-6} mol/L of DXM were added 24h before the flow cytometric analysis of Flt3 receptor expression.

Flow Cytometric Analysis of Flt3 Receptor Expression

Expression of Flt3 receptor on human leukemia, lymphoma and myeloma cell lines was determined by flow cytometric analysis. The cells were washed 3 times with PBS and counted. They were then adjusted to an appropriate concentration in a microtiter plate. The cells were incubated for 30 min with the anti-Flt3-specific IgG1 monoclonal antibody. An isotype irrelevant antibody was used as a negative control. The cells were washed and incubated with a fluoresceinated (fluorescein isothiocyanate [FITC]) goat-anti-mouse IgG. After washing, the cells were fixed in 1% paraformaldehyde PBS and analyzed on an EPICS flow cytometer (Coulter Electronic).

Proliferation Assay of Malignant Hematopoietic Cells

The effect of rhFL on malignant hematopoietic cell lines was tested. The cells were plated in 96-well plates at 5×10^3 cells/well in 150μ 1 of RPMI 1640 containing 10% FCS with or without 0 to 100 ng/ml of rhFL. 1.5% Wehi supernatant was added as a source of IL-3, required for the growth of the Baf cells. Ten ng/ml of GM-CSF, 1ng/ml of IL-6 and 0.5ng/ml of IL-11 were also added to TF1; XG-1, XG-6, XG-7 and B9-11(B9) cell lines, respectively. After 72h of culture, proliferation was measured by the microculture tetrazolium (MTT) assay as described by Carmichael et al.[7]

Statistical Analysis

Student's *t* test was used to compare the variance between two different groups. The level of significance was set at a probability of 0.05 to be considered significant.

RESULTS

Flt3 Receptor Expression on Malignant Hematopoietic Cell Lines

A total of 18 leukemia, lymphoma and multiple myeloma cell lines were screened for Flt3 receptor expression by flow cytometric analysis. The anti-Flt3-specific IgG1 monoclonal antibody SF1.340 was used to recognize the extracellular epitope of the Flt3 receptor. Of the 18 cell lines tested, 5 contained Flt3 receptor-positive cells. The levels of Flt3 receptor expression were different in the positive cell lines. In Raji, Daudi and 8266 cells, a high level of reactivity with anti-Flt3 antibody was seen, whereas HL-60 and XG6 cells showed relatively low fluorescence intensity (Table 1, Figure 1).

Effect of TNFα or DXM on the Expression of Flt3 Receptor

Following incubation with 20 ng/ml TNFα for 24h, the number of Flt3 receptor positive cells were decreased in Raji and 8266, increased in HL-60 and XG-6, and no difference in Daudi cells. After incubation with 10^{-6} mol/L DXM for 24h, the number of Flt3 receptor positive cells decreased in all the 5 Flt3 receptor positive cell lines (Table 1, Figure 1).

Effect of rhFL on the Proliferation of Malignant Hematopoietic Cells

rhFL at concentrations from 10 ng to 100 ng/ml stimulated the proliferation of Raji and HL-60 cells ($P<0.01$), but had no effect on Jurkat, U937, KG1-α, U266, TF1, Baf, B9-11, XG-1, XG-7, HSB2, JJhan,

Sub T1, Daudi, 8266 and XG6 cell lines (P>0.05). Interestingly, FL elicited a significant growth response in HL-60 cells that expressed F1t3 only weakly (Figure 2).

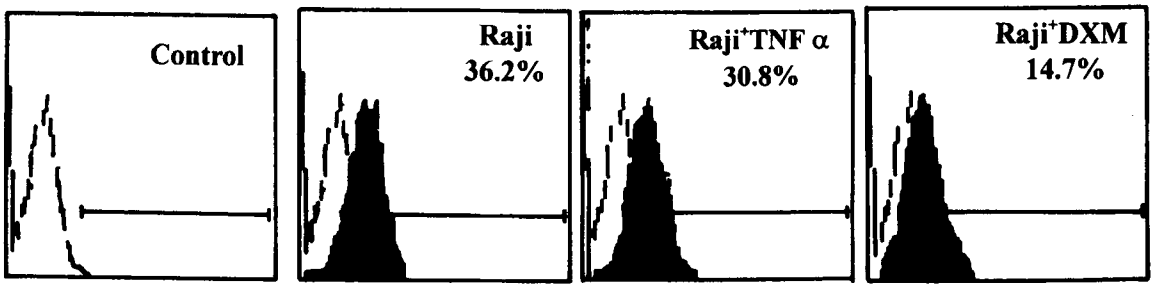

Fig. 1. Flow cytometric detection of F1t3 receptor expression on the surface of Raji cells

Table 1. Expression of F1t3 receptor in malignant hematopoietic cells

Cell Line	Cell type (original disease)	F1t3$^+$cells (negative control) (%)	F1t3$^+$cells (%)	F1t3+cells after incubation with TNFα (%)	F1t3$^+$cells after incubation with DXM (%)
Raji	Lymphoid (B type)	2.1	36.2	30.8	14.7
Daudi	Lymphoblastoid (B type)	2.7	13.4	12.6	6.7
Sub-T1	Lymphoid (T type)	0.7	0.4	0.9	0.5
JJhan	Lymphoid (T type)	1.4	1.3	1.8	1.2
HSB2	Lymphoid (T type)	0.8	1.2	1.7	1.2
Jurkat	Lymphoid (ALL T)	0.8	0.7	0.5	1.2
HL-60	myeloid (AML M2)	1.1	4.1	10.5	4.9
KG-1α	myeloid (AML)	1.6	1.8	1.5	1.2
TF-1	Erythroid (AML M6)	1.8	2.5	3.0	2.8
U937	Monocytic (Histocytic lymph)	1.7	3.8	1.9	1.9
8266	myeloma	0.7	45.5	34.2	22.8
U-266	myeloma	2.0	3.8	6.6	2.2
XG-1	myeloma	1.8	1.7	1.5	1.5
XG-6	myeloma	2.5	6.3	12.6	4.3
XG-7	myeloma	1.9	3.0	4.1	4.0
B9	Murine lymphoid (B type)	1.8	1.4	1.9	1.2
B9-11	Murine lymphoid (B type)	2.2	1.9	2.0	2.0
Baf	Murine Pro B-cell	1.1	1.2	1.3	1.2

DXM: Dexamethasone. A level of 10^{-6} mol/L DXM or 20 ng/ml TNFα was used to survey the effect of DXM on the expression of F1t3 receptor on the surface of malignant hematopoietic cell lines. Cells were stained with the specific anti-F1t3 receptor monoclonal antibody SF1.340. An irrelevant antibody of the same isotype was used for the background staining (negative control).

DISCUSSION

FL acting through its tyrosine kinase receptor F1t3 has pleiotropic and potential effects on hematopoietic cells. It can stimulate the proliferation and colony formation of human hematopoietic progenitor cells, i.e. CD34$^+$ cord blood cells, bone marrow and fetal liver cells. Synergy was reported for co-stimulation with G-CSF, GM-CSF, M-CSF, IL-3 and SCF.[8] The well-documented involvement of this ligand-receptor pair in physiological hematopoiesis raised the question whether FL and F1t3 receptor also have a role in the pathobiological of leukemia. Several investigators have focused their attention on this subject. But there were many disputes. Drexler[9] found that the vast majority of primary acute myeloid leukemia (AML) cells and continuous human myeloid cell lines were F1t3-positive. Among the myeloid cell

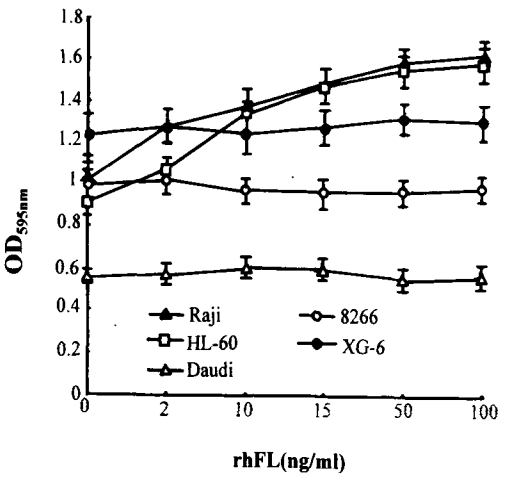

Fig. 2. Proliferative effect of rhFL on malignant hematopoietic cell expressed the Flt3 receptor

.lines, predominantly the monocytic and myelocytic cell lines were Flt3-positive whereas the erythrocytic and megakaryocytic cell lines were Flt3-negative. They also found that some leukemia cell lines displayed both ligand and receptor, stimulated the proliferation of themselves by autocrine, intracrine and paracrine. However, Siitonen and his colleagues found that FL did not induce the growth of malignant hematopoietic cells in myeloproliferative disorders.[10] In this report, we found that Flt3 receptors were expressed on the surface of certain cell lines derived from B type lymphoma (Raji and Daudi), multiple myeloma (8266 and XG-6) and acute myeloid leukemia (HL-60). There was however no obvious correlation between the stimulator effects of FL and the occurrence of Flt3 receptor; Thus Raji (high Flt3) responded, but so did HL-60 (low Flt3). Most cell lines, such as Jurkat, U937, KG1-α, U266, TF1, Baf, B9-11 XG-1, XG-7, HSB2, JJhan and Sub T1 cells showed neither significant expression of Flt3 receptor nor a response to FL.

In some Flt3-positive cases, e.g. Daudi, 8266 and XG6 cells, there was also no response to rhFL. There are four possibilities:[11,12] (1) the cells may be truly non-responsive; (2) the cells may be producing endogenous ligand (and are thus refratory to exogenously added factor); (3) in certain cases the growth factor drives not proliferation, but differentiation; and (4) the growth factor inhibits apoptosis thus promoting survival. Therefore, a considerable exploration must be taken when an acute leukemia blast has not response to the stimulation of FL.

This suggests that FL may be used safely in many cases of hematopoietic malignancies and may not cause the proliferation of malignant cells. But the Flt3 receptor must be surveyed continuously in patients with B type lymphoma and acute myeloid leukemia when FL was considered to be used.

We also found that the expression of the Flt3 receptor in the 5 Flt3 receptor-positive cell lines decreased after the cells were incubated with 10^{-6} mol/L DXM. But the effect of TNFα on the expression of Flt3 receptor was not obvious. It might be clinically relevant to combine FL with DXM in Flt3 receptor positive hematopoietic malignant patients in order to down-regulate the expression of Flt3 receptors and to inhibit the proliferation induced by FL when FL was used in the stimulation of hematopoiesis and anti-tumor biotherapy. It must be stressed, however, that we carried out our study with leukemia, lymphoma and myeloma cell lines *in vitro*; it is essential to study the effects of the recombinant FL on fresh tumor samples and to survey closely its effects in vivo.

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