Journal of the Egyptian Society of Haematology & Research, Vol. 5, No. 1, March: 29-34, 2009

Does Isoform IL-10δ3 Expression Have a Protective Role in Pediatric Precursor B-ALL?

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ABSTRACT

Background: Precursor B-acute lymphoblastic leukemia (precursor B-ALL) is the most common form of cancer in children.

The prognostic factors in pediatric precursor B-ALL mostly include clinical and biological characteristics that are assessable at diagnosis as well as early treatment response and outcome. Full-length IL-10 expression and the splicing-derived IL-10 variant (termed IL-10δ3) were detected in relapsed ALL samples and had an impact on disease outcome.

Aim: The purpose of our study was to evaluate whether the expression of IL-10 and IL-10 isoform, IL-10δ3, could be of prognostic relevance in childhood precursor B-ALL.

Patients and Methods: Fifty eight children (age 0.9 to 21 years) with precursor B-ALL were included in the study. They presented to the pediatric oncology department at NCI, Cairo University, during the time period from the January 2003 to December 2005. Reverse-transcription polymerase chain reaction was done on bone marrow and/or peripheral blood samples to detect IL-10 expression and/or its isoform IL-10δ3.

Results: Within the total number of 58 patients, full-length IL-10 and/or IL-10δ3 isoform transcripts were expressed in 34/58 patients (58.6%), 23/58 cases (39.6%) expressed isoform IL-10δ3, 19 cases had full length IL-10 and IL-10δ3 isoform simultaneously while 4 patients had Isoform IL-10δ3 as the sole variant. Patients expressing IL-10δ3 had a significantly longer EFS (p=0.005).

Conclusion: IL-10 isoform IL-10δ3, expression was associated with a favorable prognosis, decreased incidence of relapse in first complete remission and significantly better event-free survival in pediatric precursor B-ALL.

Key Words: *IL-10 – IL-10δ3 – Precursor B-ALL.*

INTRODUCTION

Interleukin-10 (IL-10) is a pleiotropic; homodimeric cytokine produced by a large variety of cells, including monocytes/macrophages, B and T lymphocytes, and resident brain cell populations such as microglia and neurons **[1]** and may act as a cancer-promoting agent **[2]**. Classically, IL-10 is considered a cytokine with a wide range of immunosuppressive and anti-inflammatory activities via the inhibition of lymphocyte and monocyte function and the secretion of inflammatory cytokines **[3]**.

The IL-10 gene comprises 5 exons, spans ~5.2KB, and is located on chromosome 1 at 1q31-1q32 **[4]**. In addition to the full-length IL-10, there is a splicing-derived IL-10 variant that lacks the entire exon 3, termed IL-10δ3. The in-frame splice variant resulting from skipping of exon 3 does not cause alteration of the translational reading frame **[5]**. The exact function of IL-10 isoform, IL-10δ3, is largely unknown. Although most splicing derived isoforms have not been functionally defined, some have been shown to possess antagonistic activities and to act competitively with the native cytokines and receptors **[6-8]**.

Several studies have demonstrated that IL-10 was associated with therapy outcome in haematological and non hematological malignancies **[9-16]**.

IL-10 has been detected in the leukemic cells of most ALL and AML cases and was reported to suppress the immune reactions, suggesting that IL-10 could be associated with escape of leukemia cells from immune surveillance **[5,17-19]**.

Precursor B-acute lymphoblastic leukemia (precursor B-ALL) is the most common form of cancer in children **[20]**.

The prognostic factors in pediatric precursor B-ALL mostly include clinical and biological characteristics that are assessable at diagnosis

as well as early treatment response and outcome. IL-10 might correlate with clinical outcome in childhood acute lymphoblastic leukaemia (ALL). Full-length IL-10 expression and the splicing-derived IL-10 variant (termed IL-10δ3) were detected in relapsed ALL samples and had an impact on disease outcome **[5]**.

In this study we evaluated the expression of IL-10 and its IL-10 isoform IL-10δ3 in 60 children with precursor B ALL to verify its potential prognostic relevance.

PATIENTS AND METHODS

This was a retrospective study done on samples available for pediatric patients. Patients were children with precursor B-ALL presenting to the Pediatric Oncology Department at NCI, Cairo University, during the time period from the January 2003 to December 2005. The study included 58 newly diagnosed patients. They were 36 male and 22 female with age ranging from 11 months to 21 years with a median of 6 years including 2 infants 11 months each. Written informed consent was obtained from the patients' parents and the protocol was approved by the Institution Research Board of the NCI, Cairo University.

All patients were subjected to full clinical, radiological and laboratory investigations including chest X-Ray, abdominal Ultrasound, CSF examination and a full chemistry profile including liver and kidney function tests, complete blood count (CBC) and bone marrow aspiration.

Patients were diagnosed according to standard methods including blood picture, bone marrow, cytochemistry and immunophenotyping.

Immunophenotyping was done using monoclonal antibodies; stained cells were analyzed on Coulter XL. The Panel included CD1, CD2, CD3, CD4,CD5, CD7, CD8, CD10, CD19, CD22, Cytoplasmic μ, anti κ, anti λ, CD13, CD33, anti class II MHC and TdT **[21]**.

RNA extraction, reverse transcriptase polymerase chain reaction (RT-PCR):

Peripheral blood and/or Bone marrow samples collected on EDTA for RNA preparation were obtained at the time of diagnosis for the 58 newly diagnosed patients.

Total RNA was extracted from patients' samples using total RNA isolation kit, (Purescript, Gentra Minneapolis USA) according to the manufacturer's instructions. RNA was stored at –80°C until tested.

Quantification and purity of RNA were determined by measuring the absorbance at 260nm (A260) and (A280) using a spectrophotometer (nanodrop). Pure RNA of an A260/A280 ratio of 1.9-2.1 was used. The integrity and size distribution of total RNA purified (18S and 28S) was checked by running on 1% agarose gel electrophoresis for 20 minutes at 80 volts and ethidium bromide staining. The GeneAmpGold RNA PCR Reagent Kit (P/N 4308206 Applied Biosystems) was used for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification from total RNA was performed according to the manufacturer's instructions.

First-strand cDNA was synthesized from 2μg of RNA using RT reaction. The integrity of cDNA for all cases was tested by amplifying the house keeping gene, β actin.

For the detection of IL-10 and its splice variant, the following primer pair was used **[5]**, IL-10 sense primer: 5'ATGCACAGCTCAGCACTGCT 3'; IL-10 antisense primer: 5'TCAGTTTCGTATCTTCATTGTCAT3'. Amplification was done in the Thermal Cycler, cycle conditions were as follows: Initial denaturation at 94°C for 10 minutes, followed by 35 cycles at 94°C for 45 seconds, 62°C for 30 seconds, 72°C for 1.25 minutes, and a final elongation at 72°C for 10 minutes. The amplification products were separated on 2% agarose gel electrophoresis at 100 volt for 20 minutes and visualized by ethidium bromide staining (Fig. 1). All assays were done in duplicate. BM samples negative for IL-10δ3 expression were confirmed by nested PCR.

Treatment:

All enrolled patients were treated according to NCI protocols modified from the St. Jude Total XIII protocols. Infants (≤12 months) were treated according to infantile protocol (interfant 99).

Follow-up time:

Patients were followed-up for a median follow-up time of 35 months (range 6 month to 72 months).

Statistical analysis:

Frequencies were calculated for descriptive purposes. Differences in the distribution of categorical variables were analyzed by χ^2 or Fisher's exact test if the frequencies were small (<5) and by Mann-Whitney U for non-paired data test. To verify the hypothesis of differences in mean values among the independent groups the ANOVA analysis of variance was used. Analysis of the probabilities of event-free survival (pEFS) was performed using the Kaplan-Meier method and the groups were compared with the log-rank test. Statistical analysis was done by the SPSS Software for Windows (version 18; portable SPSS). Significance was set to $p<0.05$ and highly significant to $p<0.01$.

RESULTS

IL-10 was detected at approximately 540bp, while isoform IL-10δ3 at 385bp.

The phenotype of the 58 cases was 4 pro B, 28 CALL and 26 pre B.

Full-length IL-10 and/or IL-10δ3 isoform transcripts were expressed in 34/58 patients (58.6%), 23/58 cases (39.6%) expressed isoform IL-10δ3 (Table 1); 19 cases had full length IL-10 and IL-10δ3 isoform simultaneously while 4 patients had Isoform IL-10δ3 as the sole variant (Table 2).

There was no statistically significant correlation between IL-10 expression and any of the tested parameters, age, sex, TLC, Immunophenotype, liver and/or spleen size or response to therapy.

Although not statistically significant, cases expressing IL-10δ3 showed less incidence of relapse compared to patients expressing only the full length IL-10 transcript and/or patients not expressing IL-10 at detection limit (p= 0.267). A total of 11/58 patients relapsed after achieving complete remission. They were 6/24 (25%) IL-10 negative, 3/11 (27.3%) expressing the full length IL-10 and 2/23 (8.7%) patients expressing the IL-10 and the IL-10δ3 variant. None of the 4 patients expressing IL-10δ3 as the sole variant relapsed.

Event free survival (EFS):

Patients expressing IL-10δ3 had a significantly longer EFS (p=0.005) (Table 1). The 4 cases expressing IL-10δ3 as a sole variant, all had an event free course (range 27-70 months) (Table 2).

In survival curve analysis of predicted event free survival (pEFS) (Fig. 2), patients with IL-10δ3 expression had a significantly better pEFS at five years (p=0.047) compared to patients expressing only the full length IL-10 transcript or patients expressing IL-10 below detection limit (IL-10 negative) (p=0.034 and 0.026, respectively). Also, when comparing non expressing IL-10δ3 to IL-10δ3 expressing cases, pEFS significance became more marked (p= 0.014) at five years (Fig. 3).

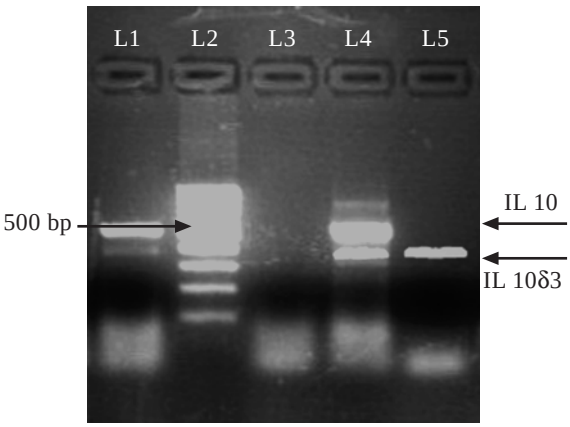

Fig. (1): Expression of IL-10 and isoform IL-10δ3 in precursor B-ALL.

Lane 1 : IL 10 positive case (540bp).
Lane 2 : 100 bp molecular weight marker (reference band 500bp).
Lane 3 : Negative control.
Lane 4 : IL 10 positive and IL 10δ3 positive case (540bp and 385bp).
Lane 5 : IL 10δ3 only positive cases (385bp).

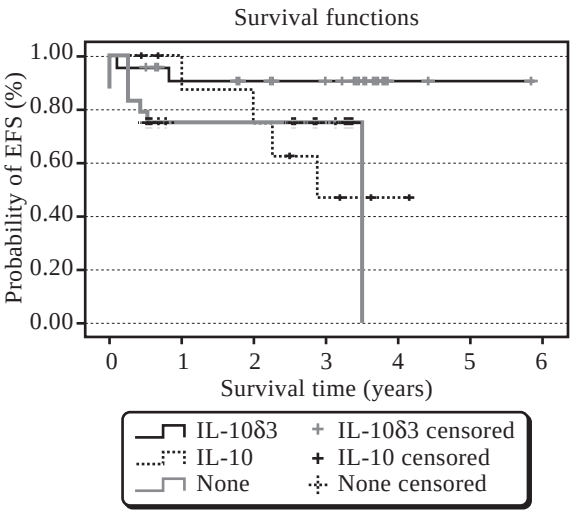

Fig. (2): Correlation between IL-10 expression and pEFS in pediatric precursor B-ALL patients.

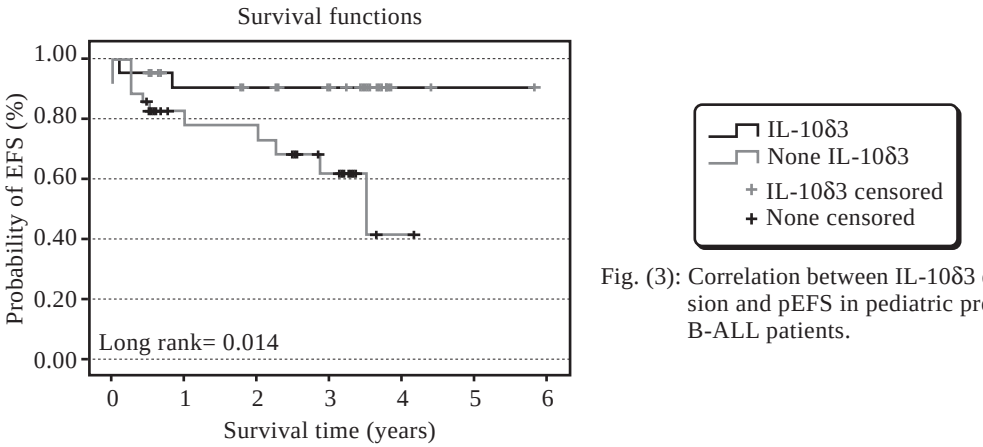

Fig. (3): Correlation between IL-10δ3 expression and pEFS in pediatric precursor B-ALL patients.

Table (1): Correlation between event free survival and IL-10 expression in precursor B-ALL patients.

	IL-10 negative	IL-10 full length	IL-10δ3 isoform	Total	*p* value
Number (%)	24(41.4%)	11 (18.9%)	23 (39.7%)	58 (100%)	
EFS (months)					
median	8	27	41		0.005*
Range	(0-32)	(5.4-30.5)	(6-70)		

IL: Interleukin,
* : Statistical significance: $p \leq 0.05$.

Table (2): Features of the 4 precursor B-ALL patients expressing IL-10δ3.

	IL-10δ3			
	Case 1	Case 2	Case 3	Case 4
Age (years)	10	8	11	6
Sex	F	M	M	F
TLC ($x10^9$/L)	6.77	101	67	57.47
Liver below costal margin (cm)	2	Free	2	Free
Spleen below costal margin (cm)	3	Free	6	Free
CD34 positivity	Yes	Yes	Yes	Yes
CR	Yes	Yes	Yes	Yes
Relapse	No	No	No	No
Status	alive	alive	alive	alive
EFS (months)	27	44.13	43.66	70
OS (months)	27	44.13	43.66	70
Others	t(4;11)	none	none	none

IL : Interleukin. EFS: Event free survival.
CR: Complete remission. OS : Overall survival.

DISCUSSION

IL-10 is an immunoregulatory cytokine and its main biological function is limitation and termination of inflammatory responses. IL-10 also regulates differentiation and proliferation of several immune cells **[22]**. Antiangiogenic properties of IL-10 have also been described **[23]**. Thus, its dual role as immunosuppressive and antiangiogenic cytokine may have both promoting and inhibiting effect on tumor development and progression **[24]**.

Studies confirmed the secretion of IL-10 by leukemic cells in bone marrow specimens of children with relapsed ALL **[18]**. Also, pretreatment serum levels of IL-10, IL-12 and IL-10/IL-12 balance in children with Soft Tissue Sarcoma, Hodgkin's Lymphoma and ALL might be of value as additional prognostic tools to

predict the response to therapy and probability of event free survival (EFS) and overall survival (OS) **[25]**.

Results in AML were contradictory. A report proposed that IL-10 contributed to T-regulatory (Treg) mediated suppression; Treg were reported to control peripheral immune tolerance and their accumulation in the peripheral circulation of AML patients mediate vigorous suppression. Hence, patients with lower Treg frequency at diagnosis were shown to have a better response to induction chemotherapy **[16]**, while in the other, IL-10 was not found to be correlated with CR, survival, or EFS **[14]**.

The involvement of IL-10 expression in childhood ALL has been suggested in various studies **[5,26,27]**. Also, published reports connected genotype of IL-10 with sensitivity for steroid therapy and proposed to include this feature into prognostic factors, especially in children with disease relapse **[26,27]**. IL-10 gene polymorphism studies suggested a correlation between the IL-10 genotype and prednisone response in childhood ALL. Patients displaying the IL-10 G/G genotype might have a lower risk for poor prednisone response **[12]**.

Similar to our findings other reports suggested that the splicing-derived IL-10 isoforms may modulate IL-10-mediated biologic effects and therapeutic efficacy in lymphatic disease and expression of IL-10δ3 is a positive prognostic feature in childhood ALL **[27]**. Our data showed a possible modulatory role of IL-10δ3 particularly in the decreased incidence of relapse. Cases expressing IL-10δ3 showed less incidence of relapse compared to patients expressing only the normal IL-10 transcript and/or patients expressing IL-10 below detection limit. In our study, none of the 4 patients expressing IL-10δ3 as a sole variant relapsed. Interestingly, one of the cases was a pro B, harboring t(4;11) rearrangement, a situation that is known to be of poor prognosis, yet the patient had an event free course, EFS and OS of 27 months. However, the follow-up period is not long enough to exclude the possibility of relapse at a later point of time.

In conclusion, we report that in childhood precursor B-ALL, IL-10 isoform, IL-10δ3, expression might be associated with a favorable prognosis, decreased incidence of relapse in first complete remission and statistically better event-free survival. Screening of a larger group of patients is needed to confirm, the unfavorable impact of the lack IL-10 isoform IL-10δ3 expressions and if its presence might have a protective role, so therapy and management of patients would be modified accordingly.

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