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S-phase Fraction and Pro-inflammatory Cytokine Expressions in Acute Lymphoblastic Leukaemia

[1]Maha A, [2]SK Cheong, [2]CF Leong & [1,3]HF Seow

[1]Dept of Clinical Lab Sciences, Faculty of Medicine and Health Science,
[3]Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
[2]Clinical Haematology & Stem Cell Transplantation Services,
Hospital Universiti Kebangsaan Malaysia

ABSTRACT

Objective: Despite much progress in treatment strategies, long term survival of adult ALL is still inferior to that in children. The underlying mechanisms for these differences are largely unknown. Intensification of contemporary therapy has also resulted in many children being over-treated. The action of chemotherapeutic drugs used in the treatment of ALL includes cell cycle dependent agents which are effective on cells that are proliferating. Cell proliferation in haemopoietic cells is controlled by cytokines. Thus, we proposed to study the cell cycle profile of ALL cases and also expression of cytokines to determine their role in affecting treatment outcome in the different age groups. **Methods:** We determined the S-phase fraction from the cell cycle profile by flowcytometry and tested the expressions of cytokine IL-1β, IL-6, IL-18, IFN-γ, TNF-α and GM-CSF using RT-PCR in d*e novo* ALL cases. **Results:** We found a significantly higher S-phase fraction in samples from children 2-10 years old compared to the older age group (>10 years old) (p=0.001). GM-CSF was found to be expressed in a significantly lower percentage of children compared to adults (p=0.008). **Conclusion:** Our results implied that GM-CSF may have induced cell cycle arrests in adult ALL resulting in a lower percentage of S-phase fraction. This may contribute to the poorer prognosis in adult ALL because non-cycling blasts are less sensitive to some chemotherapeutic drugs.

Keywords: ALL, S-phase fraction, GM-CSF, age

INTRODUCTION

The prognosis for children with acute lymphoblastic leukaemia (ALL) has improved dramatically over the past four decades. Contemporary therapy has focused on risk adapted therapy based on prognostic factors and intensifying treatment using established agents that have been documented, rather than the introduction of new drugs. Success however, is critical to the accurate assignment of individual patients to specific risk groups. Unfortunately, this is a difficult and expensive process requiring a variety of laboratory studies including morphology, immunophenotyping, cytogenetics and molecular diagnostics.[1] Furthermore, intensified treatment is compounded by an increased risk of therapy-related secondary malignancy.[2] ALL is now curable in 60-80% of children but adults adopting similar treatment protocols obtain disease free survival in only 30-40% of patients.[3] Furthermore, despite these improvements, many children are being over-treated, while subgroups of children still do poorly.[1]

Treatment outcome is also dependent on the underlying biology of the blood cancer and the host.[1] Drugs used in acute leukaemia are active at different phases of the cell cycle, thus their combination and sequential use make them very effective in the various stages of treatment.[4] In ALL, drugs such as glucocorticoids inhibit proliferation and arrest cell cycle to induce apoptosis[5] while vincristine and methotrexate are cell cycle specific agents [4]. More intensive drugs such as daunorubicin are used on non-proliferating blasts cells. The proliferation rate of blasts cells determined by S-phase fraction may be useful as a prognostic marker to predict treatment outcome. ALL samples were observed to have a higher S-phase fraction than acute myeloid leukaemia (AML) samples in paediatric acute leukaemia. Increased proliferation was also associated with increased *in vitro* sensitivity to several anticancer agents in d*e novo* ALL.[6]

Few reports were available on the constitutive expression of cytokines in ALL. Interleukin (IL)-1β and tumour necrosis factor (TNF)-α were reported to be expressed in a substantial subset of patients with B-cell derived ALL (n=14).[7] Granulocyte-macrophage colony-stimulating factor (GM-CSF) gene, however, was not expressed in "common" (pre-B cell) ALL (n=11)[8] and IL-6 mRNA transcripts were also not found in ALL of B-cell origin.[9]

We determined the cell cycle profile and constitutive expression of cytokines in d*e novo* ALL to understand the biology of the cell that may be a factor in influencing drug response.

MATERIALS AND METHODS

Patient Sample

Bone marrow or peripheral blood sample was collected with patient consent from the haematology wards of Hospital Universiti Kebangsaan Malaysia. Selection criterion was patients diagnosed with d*e novo* acute lymphoblastic leukaemia. Exclusion criteria was patients who has undergone treatment.

Cell Isolation

Mononuclear cell isolation was performed by gradient density centrifugation on Ficoll-Hypaque (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Cells were washed to remove excess Ficoll-Hypaque. A wash constituted of diluting cells with excess PBS followed by a spin at 1000 rpm for 10 min to obtain cell pellet. Supernatant was decanted. Cells were determined by the tryphan blue exclusion test to be at least 70% viable before further processing.

Cell Permeabilization and DNA Staining

Cell pellet was re-suspended in PBS containing 2% formaldehyde and left at 4°C for half an hour to fix. An equal volume of 0.4% of Triton-X (in PBS) was added and incubated for 5 min at 4°C. At the end of permeabilisation, cells were washed and re-suspended with fresh PBS to obtain a cell concentration of $5x10^6$ cells/ml. After washing, permeabilised cells were incubated with 7-AAD (7-amino-actinomycin D) at 50 µg/ml for 30 min at 4°C in the dark. At the end of incubation, 400 µl PBS was added and samples were sent for analysis on a FACSCalibur (Becton Dickinson, USA). 7-AAD was detected in the far red range of the

spectrum (650 nm long pass filter) on the FL3 detector. Cell cycle profile was generated on the CellQuest software (Becton Dickinson, USA).

RNA Isolation

RNA was isolated according to the manufacturer's instructions (Tri-Reagent, Molecular Research, USA). Briefly, 1 ml of Tri-Reagent was added to the cell pellet containing 2 million cells. To this mixture, 0.2 ml of chloroform was added. After gentle shaking the tube was left to stand in room temperature (RT) for 2-15 min followed by centrifugation at 12,000 rpm for 15 min at 4°C. The upper aqueous phase which contains RNA was transferred into a new tube and mixed with 0.5 ml isopropanol. The RNA was precipitated and centrifuged at 12,000 rpm for another 8 min at 4°C. Isopropanol was discarded and replaced with 75% ethanol. After a second wash with cold ethanol and a second spin, the RNA pellet was left to dry briefly. RNA was dissolved in an appropriate amount of diethylpyrocarbonate (DEPC) treated ultra-pure water containing RNase inhibitor (0.1 U/μl) and stored at –80°C.

The purity and quantity of RNA were determined on a spectrophotometer. The ratio of wavelength reading at 260:280 *nm* was between 1.8-2.0. The RNA concentration was estimated by measuring absorbance at 260 *nm*.

Reverse Transcription (RT)

In a sterile RNase-free microcentrifuge tube, 2 μg RNA was added to 0.5 μg of oligo$(dT)_{15}$ primer (0.5 μg/ml) (Promega, USA) and DEPC-treated water to make a total volume of 9 μl. The tube was heated at 70°C for 5 min. After heating, the tube was cooled on ice for another 5 min. to prevent secondary structure from reforming, and centrifuged briefly to collect the solution at the bottom of the tube. A cocktail of the following reagents were made: 4 μl 5x reaction mix, 1 μl dNTP mix (10 mM) (Fermentas, USA), 0.5 μl RNase inhibitor (40U/ml) (Promega, USA) and DEPC-treated water to make up total volume of 9 ml. The cocktail was added to tubes containing RNA and oligo$(dT)_{15}$ primer and incubated at 37°C for 10 min. Two μl of M-MLV RT (200 U/ml) (Promega, USA) was added and then incubated for another 2 hours at 37°C. At the end of incubation, the tube was again heated at 70°C for 5 min followed by chilling on ice for 5 min. RT products were stored at –20°C.

Polymerase Chain Reaction (PCR)

Two μl of first strand cDNA was used for PCR amplification. The sequences of the primers used for amplification of IL-1β, IL-6, IL-18, TNF-α, interferon (IFN)-γ, GM-CSF and β-actin and the expected band size of the amplicons are as listed in Table 1. A cocktail of the following was made and added to the tube: 2 μl 10x buffer, 1 ml (0.5 μM) each of forward and reverse primers, 1-2 μl $MgCl_2$ (Fermentas, USA), 0.4 μl dNTP mix (Ferementas, USA), made up to a total of 17 μl with DEPC-treated water. Lastly, 1 μl of Taq DNA polymerase (1U/μl) (Fermentas, USA) was added. The tubes were then subjected to a hotstart which consisted of heating to 94°C for 3 min. The thermocycling conditions were: denaturation at 94°C for 1 min, annealing at 52°C for amplification of β-actin, 55°C for 18S (housekeeping genes) and 56°C for IL-1β, IL-18, IL-6, IFN-γ, TNF-α and GM-CSF (Table 2) and extension at 72°C for another 1 min. Thirty-five cycles were performed for

amplification of the cytokines, 25 cycles for 18S and 40 cycles for β-actin. At the end, a second extension at 72°C was carried out for 5 min, after which the product was left at 4°C. PCR was carried out on a Perkin Elmer GeneAmp 2400 thermocycler (Boston, USA) or a gradient thermocycler (Biometra, Germany). At the end of PCR, 10 µl of the reaction was loaded onto a 1.0 % agarose gel in TAE buffer containing ethidium bromide. PCR products were electrophoresed on the gel together with the Generuler 100 bp DNA ladder (Fermentas, USA). Bands were visualised on an imaging system utilising the GeneSnap software programme from SynGene, a division of Synoptics Ltd (Cambridge, UK).

Table 1. Primer sequences and expected amplicon sizes of the cDNA used in this study

Gene	Sequences	Band size (bp)	Reference
Cytokines:			
IL-1β	F: ACTACAGCAAGGGCTTCAGG R: CATATCCTGTCCCTGGAGGT	290	[10]
IL-6	F: ACGAATTCACAAACAAATTCGGTACA R: CATCTAGATTCTTTGCCTTTTTCTGC	335	[11]
IL-18	F: GATTACTTTGGCAAGCTTGAA R: GTCTTCGTTTTGAACAGTGAA	480	[12],
TNF-α	F: GCGAATTCCCTCCTGGCCAATGGCGTGG R: CTAAGCTTGGGTTCCGACCCTAAGCCCC	507	[11]
GM-CSF	F:GCTGCTGAGATGAATGAAC R:AGTCAAAGGGGATGACAAG	265	[10]
IFN-γ	F:GGTTCTCTTGGCTGTTACTGCC R:GTTGGACATTCAAGTCAGTTACCGA	340	[11]
Housekeeping Genes:			
18S	F: GTAACCCGTTGAACCCCATT R: CCATCCAATCGGTAGTAGCG	188	
β-actin	F: ACCGAAGCTCCAATGAATCCAAAATCC R: GTTTGGTCAATACCAGCAGCTTCCAAA	479	

Statistical Analysis

Statistical analysis was performed on SPSS Window version 10.0 statistical software. The non-parametric Mann-Whitney U test was used to compare between two groups while Kruskall-Wallis test was used to compare between more than two groups. Correlation between the values of two groups was analysed using the Pearson correlation test. The significant level was defined as a p value <0.05.

RESULTS

Patient Sample

A total of 22 ALL patients were included in this study. These were patients of Hospital Universiti Kebangsaan Malaysia between Jan 2000 and Sept 2002. Patients were grouped

into age group 2-10 years and age group of more than 10 years old (>10 years) [4]. Fourteen patients were of the younger age group while eight were of the older age group. Table 2 shows the biological, immunological and FAB subtypes of the ALL cases.

Table 2. Biological, immunological characteristics and FAB subtype of ALL patients

No	Ref	Age	Sex	Site	CD19	CD10	CD34	FAB
	2-10 yr							
1	32	9	M	BM	+	+	+	ND
2	71	5	M	BM	+	+	+	ND
3	91	7	M	PB	NF	NF	NF	L2
4	99	3	M	BM	+	+	+	L1
5	105	3	F	PB	+	+	+	L1
6	118	9	M	BM	+	+	+	L2
7	119	2	M	BM	+	+	+	L1
8	147	4	M	BM	+	+	+	L2
9	150	4	M	PB	+	-	NF	L1
10	159	4	M	BM	+	+	NF	L1
11	165	2	M	BM	+	+	+	L1
12	170	4	F	BM	+	+	+	L1
13	243	10	M	BM	+	+	NF	L1
14	244	9	F	BM	+	+	NF	L1
	>10 yr							
1	53	13	M	BM	+	+	+	ND
2	74	16	M	BM	+	+	+	ND
3	75	13	M	BM	+	-	+	L2
4	79	13	M	BM	NF	NF	NF	L1
5	94	12	M	PB	+	+	+	L1
6	132	23	M	BM	+	+	+	L2
7	180	25	M	PB	NF	NF	NF	L2
8	191	13	M	BM	+	-	+	L2

All cases were diagnosed based on morphological, cytochemical as well as immunological methods. Positive (+) cases had blasts expression of CD markers 20% or more. PB = peripheral blood, BM = bone marrow, ND=not done, NF=not found.

S-phase Fraction

Fig. 1 shows the cell cycle profile generated from the CellQuest software. The percentage of S-phase fraction (which is the region labeled M2) was determined from the histogram statistics. *Fig. 2* shows that a higher median was obtained for the younger age group. Percentage of S-phase fraction ranged from 2-13% and 1-2%, for the younger and older age groups, respectively. This was shown to be statistically different ($p= 0.001$).

mRNA Expression of Cytokines

GM-CSF, IL-1β, IL-6, IL-18, IFN-γ and TNF-α were expressed in 56% (n=16), 84% (n=19), 21% (n=14), 22% (n=18), 7% (n=14) and 79% (n=14) of AML cases, respectively. Percentage

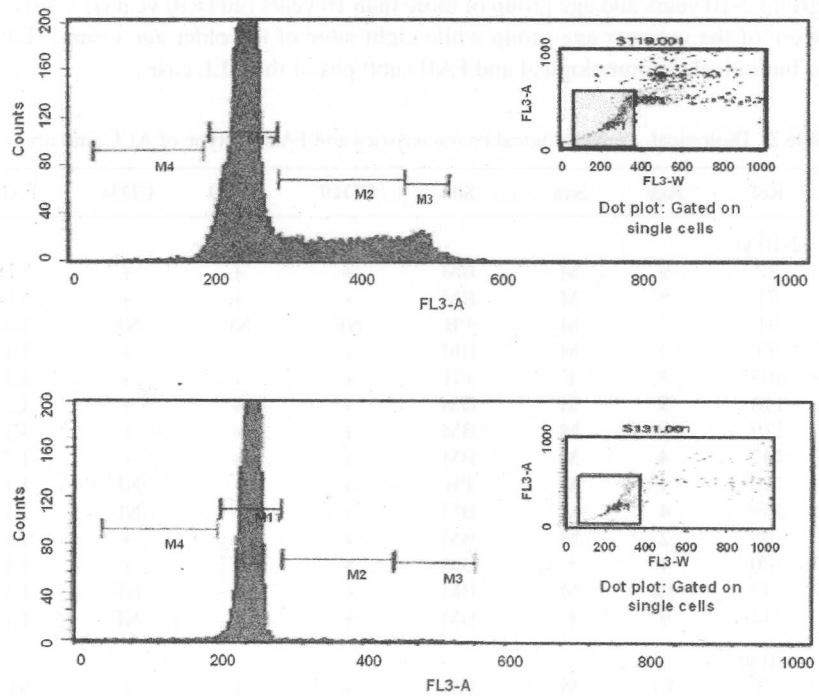

Figure 1. Cell cycle profile showing G0/G1 (M1), synthesis (M2), G2/M (M3) and sub-G0 (M4) peaks. Percentages were obtained from histogram statistics on CellQuest software. Top panel shows a case with a complete cell cycle profile indicating cells in proliferation. Bottom panel shows a case with a peak only at G0/G1, indicating cells not in proliferation

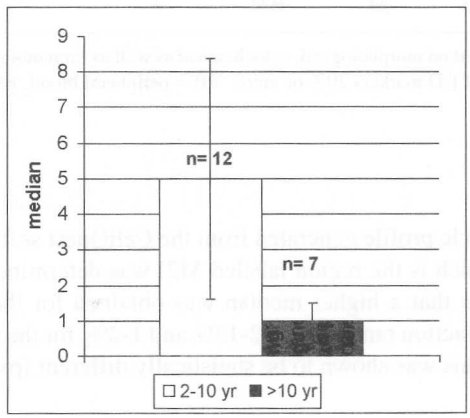

Figure 2. Median value of percentage of cells in S-phase fraction in of ALL cases and comparison between the different age groups. Not all samples were analysed for each gene because of insufficient samples. n refers to the number of cases per group

of cases expressing each gene between the two age groups is shown in *Fig. 3*. IL-1β and TNF-α were highly expressed in both age groups, while expressions of IL-6, IL-18 and IFN-γ in ALL cases were low. We found a higher number of cases from the older age group expressing GM-CSF compared to the younger age group (*Fig. 3*). The expressions of all cytokines were not significantly different between the two age groups except for GM-CSF (p=0.008). *Fig. 4* shows mRNA expression of GM-CSF and β- actin, as housekeeping gene, in ALL samples. Amplicons of the expected band sizes were observed.

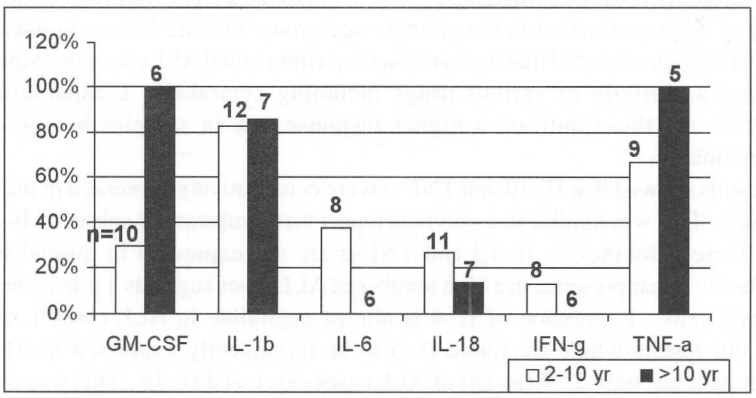

Figure 3. Percentage of cases expressing GM-CSF, IL-1β, IL-6, IFN-γ and TNF-α mRNA in the two different ALL age groups. Not all samples were analysed for each gene because of insufficient samples. n = number of cases per group

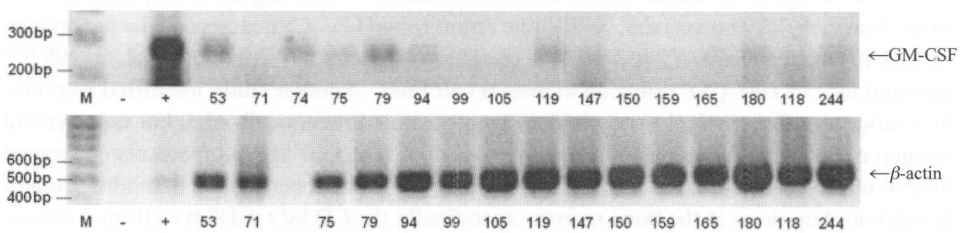

Figure 4. mRNA expression of GM-CSF and b-actin in ALL cases. Expected band size of 265 bp was observed for GM-CSF. Positive control consisted of RNA extracted from mitogen stimulated peripheral blood cells

DISCUSSION

In childhood acute leukaemia, S-phase fraction was reportedly higher in ALL cases (median 6.9%) than AML cases (median 5.3%).[6] The median value for ALL was comparable with the median percentage of S-phase fraction in our ALL cases from the younger age group (5%). High percentage of S-phase fraction in childhood ALL was also shown to correlate with DNA hyperploidy which is a favourable prognostic marker. The percentage of S-phase cells was observed to be higher in the hyperdiploid ALL samples (median, 8.5%) than in the nonhyperdiploid ALL samples (median, 5.7%).[10] We found a significantly ($p=0.001$) higher percentage of S-phase fraction in the younger age group who are known to have a better prognosis. It was also reported that S-phase fraction within initial ALL correlated significantly with *in vitro* sensitivity to various drugs including cytarabine, L-asparaginase and vincristine.[6] All these indicate a higher response rate in samples having a higher S-phase fraction.

Our results showed that IL-1β and TNF-α were constitutively expressed in the majority of ALL cases. This was similar to a previous report that a substantial subset of B-cell ALL expressed these cytokines.[7] IL-1β and TNF-α are not expressed in normal human B lymphocytes.[11] Their presence in a high number of ALL cases suggests a role in the survival of leukaemia cells. Expression of IL-6 is almost negligible in ALL cases[9] and this is similar to our results where we found IL-6 to be infrequently expressed in ALL cases. Among our patients, only 22% (n=18) of ALL cases expressed IL-18. This was in contrast to a higher percentage of 87% (n=15) in ALL cases in another report.[12] The results by these authors were however reported as a relative expression level of IL-18 in ALL cases to level of expression in normal cells. We found low levels of IFN-γ in ALL samples. IFN-g expression in ALL cases from published data was not found.

ALL blasts have been shown to have a lower response to haemapoietic growth factors compared with AML blasts, including to GM-CSF,[13] for example, GM-CSF enhanced the production of only IL-1a and IL-1b in blood cell cultures of patients with ALL in contrast to a wider diversity of cytokines found in AML cultures.[14] The degree of response in ALL cases, however, is controversial. While one report found GM-CSF increased the percentage of ALL blasts to a mean of 346% in 37% (n=19) of ALL cases,[16] Yoshida *et al.,* in 1992[19] reported lack of GM-CSF effect on human B cell lines. Another study identified response in a subgroup of patients, i.e. myeloid antigen positive precursor B-ALL but not myeloid antigen negative ALL samples.[17] The expression of GM-CSF receptor was also observed in 65% of precursor B-ALL.[18] In normal cells, GM-CSF has been shown to inhibit primary B cell lymphopoiesis in the bone marrow specifically the CD38(+)CD34(++)Lin(-) subset. It had no effect on the generation of B cells from the more immature, CD38(-)CD34(++)Lin(-) cells.[19]

The effect of GM-CSF on ALL as an autocrine or paracrine cytokine is not determined. GM-CSF was reported to be absent in ALL cells.[8] We, however, found that 56% (n=16) of ALL cases expressed GM-CSF. The level of expression, however, was low (in comparison with expression in AML cases, data not shown). The detection of GM-CSF in our cases may be due to the more sensitive technique that was used, i.e. reverse transcription-polymerase chain reaction (RT-PCR) compared with northern blot by the previous report.

Furthermore, we were able to show that a subgroup of patients, age > 10 years, expressed GM-CSF in more cases than the younger age group (p=0.008). This difference has not been reported before.

Taken together, our results showed that in the younger age group, fewer cases expressed GM-CSF and these cases have a higher S-phase fraction. In contrast, more cases from patients of the older age group expressed GM-CSF but these cases had lower S-phase fractions. This suggested that in the presence of GM-CSF, cells may have been inhibited from entering the cell cycle as has been reported in primary B cell lymphopoiesis in the bone marrow[20] resulting in low S-phase fraction. This may contribute to a lower response to chemotherapy since increased S-phase may enhance the cytotoxic effects of drugs such as Ara-C.[6] GM-CSF has also been used in clinical trials in AML cases for recruitment of leukaemia cells into cell cycle.[21] However, the results remain controversial as authors have reported heterogeneity of leukaemia cells in response to growth factors.[22, 23] Our results with ALL samples suggest a possible negative effect on the use of GM-CSF in ALL cases. However, the concentration of GM-CSF present may also need to be considered as it may have different effects on cells. So far, no clinical trial has yet to show priming of leukaemia cells with growth factors resulting in improvements in response rate or survival. Thus, more *in vitro* studies should be performed before further clinical trials with this approach.[21]

Our work with flowcytometry showed that it is a sensitive tool which is able to differentiate small subpopulations within a sample, enabling it to separate cells of different sub-fractions in the cell cycle. Thus, it may become a powerful tool in the study of cancer.

CONCLUSION

We observed a significantly higher percentage of S-phase fraction in ALL cases from the younger age group and also found a significantly lower number of cases from this group expressing GM-CSF. The reverse was observed in the older ALL patients. The results implied that GM-CSF expression in ALL cells may be a mechanism that inhibited cells from entering the cell cycle resulting in a low percentage of S-phase fraction. Low percentage of S-phase fraction resulted in low response to chemotherapy and a poorer treatment outcome which is associated with adult ALL. Thus, high S-phase fraction and negative expression of GM-CSF may be useful as good prognostic markers in ALL. Furthermore, the use of GM-CSF to recruit ALL cells into the cell cycle should be reconsidered. On the contrary, inhibition of GM-CSF may be advocated as an additional form of treatment in ALL patients with blasts showing expression of GM-CSF.

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REFERENCES

[1] Carroll WL, Bhojwani D, Min DJ, et al. Pediatric acute lymphoblastic leukaemia. Hematology 2003; 102-131.

[2] Barnard DR, Lange B, Alozo FA, et al. Acute myeloid leukemia and myelodysplastic syndrome in children treated for cancer: comparison with primary presentation. Blood 2002;100(2) : 427-434.

[3] Styczynski J, Wysocki M. Differences in significance of drug resistance mechanisms between adult and childhood acute lymphoblatic leukemia. Haematologia 2002;32(4) : 3313-325.

[4] Catovsky D, Hoffbrand AV. Acute leukaemia in postgraduate haematology (4th ed.). Hoffbrand AV, Lewis SM and Tuddenham EGD (eds.). Oxford: Butterworth Heinemann, 1999.

[5] Bagdassarian N, Bertrand Y, Ffrench P, et al. Role of Bcl-2 and cell cycle regulatory proteins for corticosensitivity assessment in childhood acute lymphoblastic leukaemia. Br J Haematol 2000;109(1) : 106-116.

[6] Kaaijk P, Kasper GJ, Van Wering ER, et al. Cell proliferation is related to in vitro drug resistance in childhood acute leukemia. Br J Cancer 2003;88(5) : 775-781.

[7] Kurzrock R, Kantarjian H, Wetzler M, et al. Ubiquitous expression of cytokines in diverse leukemias of lymphoid and myeloid lineage. Exp Hematol 1993;21(1) : 80-5.

[8] Young DC, Wagner K, Griffin JD. Constitutive expression of the granulocyte-macrophage colony-stimulating factor gene in acute myeloblastic leukemia. J Clin Invest 1987;79(1) : 100-6.

[9] Inoue K, Sugiyama H, Ogawa H, et al. Expression of the interleukin-6 (IL-6), IL-6 receptor, and gp130 genes in acute leukemia. Blood 1994;84(8) : 2672-2680.

[10] Kaspers GJ, Smets LA, Pieters R, et al. Favorable prognosis of hyperdiploid common acute lymphoblastic leukemia may be explained by sensitivity to antimetabolites and other drugs: results of an in vitro study. Blood 1995;85(3) : 751-756.

[11] Matthes T, Werner-Favre C, Tang H, et al. Cytokine mRNA expression during an in vitro response of human lymphocytes: kinetics of B cell tumor necrosis factor-a, interleukin (IL)6, IL-10, and transforming growth factor b1 mRNAs. J Exp Med 1993;178(2) : 521-528.

[12] Zhang B, Ma X-T, Zheng GG, et al. Expression of IL-18 and its receptor in human leukemia cells. Leuk Res 2003;27 : 813-822.

[13] Mirro J Jr, Hurwitz CA, Behm FG, et al. Effects of recombinant human hematopoietic growth factors on leukemic blasts from children with acute myeloblastic or lymphoblastic leukemia. Leukemia 1993;7(7) :1026-1033.

[14] Kaminska T, Hus I, Dmoszynska A, et al. Cytokine production in whole blood cell cultures of patients with B-lineage acute lymphoblastic leukemia. The influence of granulocyte-macrophage colony-stimulating factor. Arch Immunol Ther Exp (Warsz) 2001;49(1) : 71-77.

[15] Freedman MH, Grubberger T, Correa P, et al. Autocrine and paracrine growth control by granulocyte-monocyte colony-stimulating factor of acute lymphoblastic leukemia cells. Blood 1993;81(11) : 3068-3075.

[16] Yoshida A, Ishioka C, Kimata H, et al. Recombinant human growth hormone stimulates B cell immunoglobulin synthesis and proliferation in serum-free medium. Acta Endocrinol 1992;126(6) : 524-9.

[17] Gattei V, Aldinucci D, Attadia V, et al. Human granulocyte-macrophage colony-stimulating factor supports the clonogenic growth of B-lineage acute lymphoblastic leukemias expressing myeloid antigens. Cytokines Cell Mol Ther 1997;3(3) : 141-151.

[18] Drach D, Estrov Z, Zhao Z, et al. Granulocyte-colony stimulating factor, granulocyte-macrophage colony-stimulating factor, PIXY-321, stem cell factor, interleukin-3, and interleukin-7: receptor binding and effects on clonogenic proliferation in acute lymphoblastic leukemia. Leuk lymphoma 1994;16(1-2) : 79-88.

[19] Muench MO, Humeau L, Paek B, et al. Differential effects of interleukin-3, interleukin-7, interleukin 15, and granulocyte-macrophage colony-stimulating factor in the generation of natural killer and B cells from primitive human fetal liver progenitors. Exp Hematol 2000;28(8) : 961-73.

[20] Dorshkind K. In vivo administration of recombinant granulocyte-macrophage colony-stimulating factor results in a reversible inhibition of primary B lymphopoiesis. J Immunol 1991;146(12) : 4204-8.

[21] Schiffer CA. Hematopoietic growth factors as adjuncts to the treatment of acute myeloid leukemia. Blood 1996;88(10) : 3675-85.

[22] Estrov Z, Park CH, Reading CL, et al. The effect of granulocyte-macrophage colony-stimulating factor on undifferentiated and mature acute myelogenous leukemia blast progenitors. Exp Hematol 1992;Aug;20(7) : 886-90.

[23] Van der Lely N, De Witte T, Wessels J, et al. In vitro response of blasts to IL-3, GM-CSF and G-CSF is different for individual AML patients: factors that stimulate leukaemia clonogenic cells also enhance Ara-C cytotoxicity. Ann Hematol 1994;68(5) : 225-232.